THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 118 JANUARY 1, 1937 No. 1

TRANSIENT HYPERTENSION IN RATS FOLLOWING THE EXTRAVASCULAR ADMINISTRATION OF FLUID[1]

J. Q. GRIFFITH, JR.,[2] W. A. JEFFERS AND M. A. LINDAUER

WITH THE TECHNICAL ASSISTANCE OF R. CAMPBELL AND F. JANKUSKAS

From the Robinette Foundation, the Medical Clinic of the Hospital and the Pepper Laboratory of the University of Pennsylvania

Received for publication July 29, 1936

In a previous publication (1) we reported the association of increased intracranial pressure and vascular hypertension in the rat following the intracisternal injection of colloidal kaolin. We showed that the kaolin blocked the perineural pathways which lead to the cervical (and presumably other) lymph nodes and suggested that it produced the increased intracranial pressure by interfering with resorption of cerebrospinal fluid. Since the time of this report we have been able to measure directly the pressure of the cerebrospinal fluid, using a bubble manometer without loss of fluid. We find that whereas the pressure in the normal animal is less than 100 mm. of water, it averages about 250 in animals which have become hypertensive following the intracisternal injection of kaolin. Similarly, we have found that if the cerebrospinal fluid pressure is raised acutely to 220–270 mm. of water in the normal animal by saline passed through a needle introduced into the cistern with a micromanipulator, the blood pressure will rise in a few minutes to 180–190 mm. of mercury.[3]

With the above data at hand, it seemed probable that a similar syndrome could be produced by increasing formation of cerebrospinal fluid. A method for doing this would be to give hypotonic fluids intravenously, a procedure known to raise cerebrospinal fluid pressure. However, as we wished to study changes in blood pressure this route appeared to be undesirable, for we have repeatedly confirmed in the rat the fact already well established for other animals, namely, that blood pressure is apt to

[1] Aided by a grant from the Philadelphia Heart Association.

[2] Heckscher Fellow in Medicine.

[3] Unpublished observations.

vary widely after intravenous injections. We therefore decided to give water, either by hypodermoclysis or intraperitoneally, and for comparison to inject corresponding amounts of physiologic saline.

METHOD. Normal adult albino rats were used throughout. Animals were injected with warm distilled water or physiologic saline either subcutaneously or intraperitoneally. Hematocrit and hemoglobin determinations by a colorimetric method were made 8 to 10 hours following the injection. One or more blood pressure measurements were made from 8 to 36 hours after the injection, occasionally for 72 hours. Most animals had a single determination, which was then as a rule 24 hours after injection, but many had repeated pressures. The method used was the one we have previously described (1), in which an especially designed cuff encircles the thigh while cutaneous vessels in the dorsum of the foot are visualized through the microscope. When the pressure in the cuff exceeds systolic pressure flow in the cutaneous vessels stops, to begin again when the pressure is lowered below systolic pressure. All determinations were made under ether anesthesia.

In certain animals cerebrospinal fluid pressures were obtained by cisternal puncture using a bubble manometer without loss of fluid. Only those pressures were accepted in which the bubble showed a fair respiratory excursion and in which the animal survived the procedure. Water content of the brain was measured in the usual way by determining wet and dry brain weights, the latter after heating to constant weight in an oven at 110°C. for 72 hours. It did not prove practicable to open the ventricles, which are, at least in the normal animal, potential rather than actual spaces. Hence, the wet brain weight includes an extremely small but unknown amount of intraventricular fluid. In a few animals microscopic studies of the brain were made.

RESULTS. Figure 1 shows the blood pressure distribution in 129 animals given water or saline by the methods described. It is noted that 40.5 per cent of these developed a hypertension with blood pressures ranging between 151 and 300 mm. of mercury. Contrary to our expectation, however, it occurred equally as well with physiologic saline as with water, the figures being 40 per cent for saline and 41 per cent for water. We found that animals could survive 30 cc. of water or physiologic saline per 100 grams body weight if given subcutaneously, but if given intraperitoneally they either died in a few hours in convulsions if the fluid was water or were prostrated for several days if the fluid was saline. This suggests that the difference is in the rate of absorption. It was quite usual to find that animals given water or saline intraperitoneally returned to their pre-injection weight in 24 hours, while the weight gain persisted often 48 hours or more if the subcutaneous route had been used. Also, we know that as a rule about 50 per cent of the intraperitoneal injection was out of the

peritoneal cavity after two hours. We have considered animals with blood pressures below 65 mm. of mercury as in shock. It is to be noted that this sometimes occurred after the administration of water, but never after

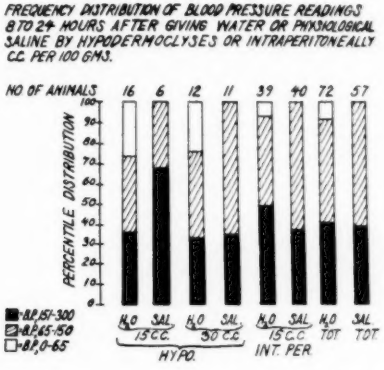

Fig. 1. Chart showing frequency distribution of blood pressure readings under varying experimental conditions.

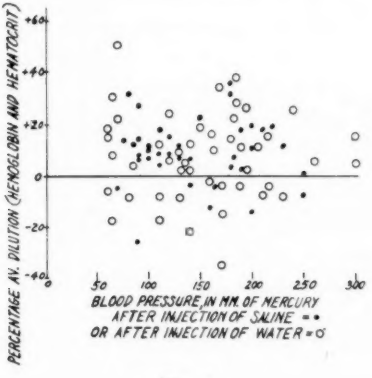

Fig. 2

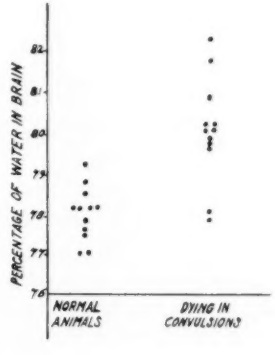

Fig. 3

Fig. 2. Chart showing lack of correlation between blood pressure in millimeters of mercury and percentage dilution of the blood.

Fig. 3. Chart showing percentage of water in brain of normal animals as compared with those dying in convulsions in "water intoxication."

saline. Animals that did not die in the first few hours as a rule recovered. In no instance did a hypertension persist longer than 48 hours.

Figure 2 shows the relation existing between blood pressure and dilution of the blood, estimated from change in hemoglobin and hematocrit deter-

minations. It is obvious that there is no correlation. While changes in hematocrit and hemoglobin were probably not precisely in proportion or perhaps occasionally not even in the same direction as one would anticipate from changes in blood volume, however, the evidence, so far as it goes, strongly suggests that dilution of the blood was not the important factor in the production of the vascular hypertension.

Figure 3 shows the percentage of water in the brain in 12 normal animals and in 12 animals dying in convulsions after 30 cc. of water intraperitoneally (i.e., in typical water intoxication). The average figure for the normal animals is 78 per cent, agreeing fairly well with the figures of Drake

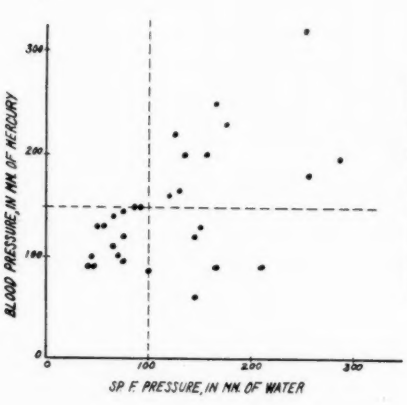

Fig. 4

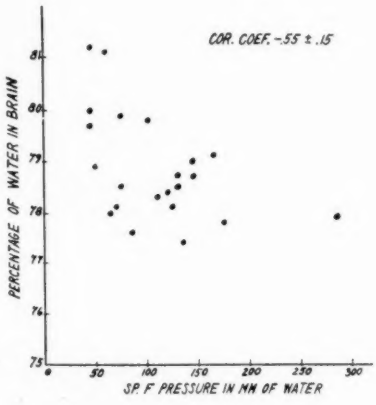

Fig. 5

Fig. 4. Chart showing correlation between blood pressure in millimeters of mercury and spinal fluid pressure in millimeters of water. The dash lines represent the top normal limits for both.

Fig. 5. Chart showing negative correlation between spinal fluid pressure in millimeters of water and percentage of water in the brain.

(2) and Donaldson (3). The average for the animals dying in convulsions is 80 per cent, an increase of 2 per cent, which agrees exactly with the figure given by Misawa (quoted by Smyth (4)) who studied water intoxication in rabbits.

Percentage water in the brain has been determined along with blood pressure determinations in 54 animals after the administration of water or saline. Of these 54 animals, 23 received water and 31 saline; 17 were hypertensive and 37 were not. No correlation was noted between the blood pressure and the percentage water content of the brain.

Figure 4 shows the relation existing between the blood pressure and the cerebrospinal fluid pressure. The dash lines mark off the top normal

limits of each. Most of the dots fall either in the rectangle of normal spinal fluid pressure and normal blood pressure or of high spinal fluid pressure and high blood pressure. Five animals showed high spinal fluid pressure with normal or low blood pressure. These five animals were among the first 12 in which these two measurements were made, and at this time we were taking blood pressures on six animals, and then determining spinal fluid pressures on the same group of six. In such experiments the time between taking the blood pressure and taking the cerebrospinal pressure was possibly an hour or an hour and a half. It is not apparent why this should have affected the spinal fluid pressure in the direction of making it too high, but it may be significant that since we changed our method so as to perform the two procedures within 2 to 3 minutes of each other, the correlation has been close. However, we have never found an animal with a vascular hypertension without an increased cerebrospinal fluid pressure. Thus, on the chart, the fourth rectangle is empty.

Figure 5 shows the relation existing between the spinal fluid pressure and the water content of the brain. It would appear that there is an inverse relationship, i.e., that a high spinal fluid pressure is apt to be associated with a normal percentage of water in the brain, and vice versa. This would not be anticipated from the correlation shown in figure 4, and the lack of correlation between the percentage water in the brain and the blood pressure as previously described. We shall take up this matter further in the discussion.

The histologic examination of sections taken from the brain showed fairly uniform widening of the perivascular spaces. There seemed to be no difference in this respect between the animals given water and those given saline.

Discussion. It is well established that the cerebrospinal fluid is formed by the choroid plexuses, with some additions from the perivascular channels of the brain. Its formation tends to be increased by conditions which lower the colloid osmotic pressure of the blood, such as occur during the absorption of extravascular deposits of fluid. It is probable, however, that because of anatomic factors involved very little increase in cerebrospinal fluid actually results.

According to the Monroe-Kellie doctrine, the cranial cavity is a closed space, the volume of which is practically constant. The volume is the sum of the volumes of brain tissue, blood, and cerebrospinal fluid. Thus, for edema of the brain to occur, blood or cerebrospinal fluid or both must be displaced. The average brain weight of the adult rat is about 1.5 gram. We do not know exactly how much blood is normally contained in the cranial cavity, but it is probably in the neighborhood of 0.2 cc., as suggested by some unpublished experiments. If this entire amount were displaced by water, the percentage increase of water in the brain would be only 2.7 per

cent, inasmuch as the blood displaced itself contains approximately 80 per cent water.

We have no figures available for the amount of cerebrospinal fluid that might be displaced by the increased size of the brain with edema. Certainly one cannot withdraw more than 0.05 to 0.1 cc. of fluid by cisternal puncture, and often the amount is much less. This represents fluid from the entire cerebrospinal space. A 3 to 6 per cent increase in water content of the brain would be required to increase the volume of the brain by 0.05 to 0.1 cc.

On the other hand, the addition of only 0.01 cc. of fluid to the cerebrospinal space, an increment that represents only 0.6 per cent of the wet brain weight, will raise the pressure of the cerebrospinal fluid from 70 to 250 mm. of water.

It will thus be seen that the first effect produced by conditions which tend to accelerate cerebrospinal fluid formation is an increased pressure, whereas changes of volume occur only later, usually near death, and even then tend to be small.

On the basis of these considerations, most of our findings can be explained. The increased cerebrospinal fluid pressure tends to produce an ischemia of the vital centers, with compensation through the raising of systemic blood pressure. Dilution of the blood is perhaps present at some time in all animals, but if the compensating factors are working well it may not be demonstrable. It is possible indeed that actual concentration may occur following the loss by diuresis of most or all of the injected fluid. Also, conditions resulting from shock may lead to concentration.

While it seems apparent that the cerebrospinal fluid pressure is the important factor in the production of a vascular hypertension, we were surprised that it varied inversely with the water content of the brain. While we have accepted no explanation for this, if we return to the closed box concept of the cranium it may be suggested that the increased cerebrospinal fluid pressure tends perhaps to prevent the development of a brain edema.

We have shown that blood pressure and spinal fluid pressure are correlated directly, that spinal fluid pressure and water content of the brain are correlated inversely, and yet blood pressure and water content of the brain are not correlated. This apparent inconsistency can be explained by the fact that the correlation coefficients of the first two relations are so low, especially that of the spinal fluid pressure and water content of the brain (correlation coefficient equals 0.55) that the third correlation might not be recognizable.

In some respects disturbance of fluid volume and composition resembles the syndrome described by Rowntree and others (5) under the name of water intoxication. Rowntree has produced this disturbance of fluid volume and composition in rats, but does not report any measurements of

blood pressure. He (6) does report some blood pressures in dogs and states that a hypertension frequently occurs, but from the protocols it appears that his dogs had been given pituitrin and were either in convulsions or in a pre-convulsive state. One chart shows a rise in pressure immediately after a convulsion. We recognize differences in the experimental syndrome we have described from that described by Rowntree, because: 1, it can be produced with equal facility with physiologic saline, which never produces "water intoxication"; 2, when water is used, relatively smaller amounts are required than were used by Rowntree. Also, the appearance of the animal is different: it almost never has convulsions and often does not appear very ill.

SUMMARY

A vascular hypertension appeared in 40.5 per cent of 129 rats given fluid, either distilled water or physiologic saline, in amounts not exceeding 30 cc. per 100 grams body weight subcutaneously or 15 cc. per 100 grams of body weight intraperitoneally. Evidence is presented that this hypertension is associated with increased pressure in the cerebrospinal fluid. A correlation with blood dilution or with increased water content of the brain could not be demonstrated.

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MOVEMENTS OF THE EYES WHEN THE LIDS ARE CLOSED

W. O. FENN AND J. B. HURSH

From the Department of Physiology, School of Medicine and Dentistry, The University of Rochester, Rochester, N. Y.

Received for publication August 3, 1936

The electrical method of recording movements of the eye, first used by Schott (1922) and Myers (1929), has been more recently studied by Mowrer, Ruch and Miller (1936), who have shown that the electrical changes, recorded by a string galvanometer from electrodes applied to the temples lateral to the eyes, are due to the polarization of the eye ball, the cornea being positive and the retina negative. This method, therefore, seemed to promise the possibility of recording movements of the eyes, even when the lids are closed.

It was first necessary to show that the method does give deflections which are quantitative measures of the deflections of the eyes. The method of calibration is illustrated in figure 1. The subject is seated about 3 meters from a blank wall directly in front of a point of fixation on the wall, *O*. Other points, *a* to *f*, are measured off to the right and the left. The subject turns his eyes from *O* to *b*, then to *a*, back to *b*, then to *a* and back to *O*. This is repeated with points *c* and *d*, and points *e* and f. A typical record of the galvanometer deflections is also shown in figure 1 (top). These deflections are measured and figures from a number of such records are averaged together. If the deflections are due simply to the polarization of the eye ball, then the potential differences resulting from a deflection of the eyes to either side of the line of fixation should be proportional approximately to the distances *Oa*, *Od*, *Oe*, etc., on the wall, or proportional to the sine of the subtended angle, α. Likewise, the deflection of the galvanometer resulting from moving the eyes from point *d* to point *c* should be equal to $2 \sin 0.5\alpha$. Data from six subjects are represented in figure 1, the galvanometer deflection in millivolts being plotted against the deflection of the eyes, expressed as $2 \sin 0.5\alpha$. The graphs are good straight lines affording therefore a strong confirmation of the theory of corneo-retinal polarization and proving that the method is a reliable one for the purpose of recording eye movements.

The slope of each of the lines in figure 1 is characteristic of the individual to which it belongs, and represents the calculated millivolt potential which would be produced by the two eyes together, if they could be deflected from a central point of fixation to a point 90° to one side of the cen-

ter, or the millivolts observed when 2 sin $0.5\alpha = 1.0$. The subjects in figure 1 gave values, from the upper graph down, of 0.84, 0.56, 0.50, 0.50, 0.40, and 0.36 millivolt. The first of these is the highest we have yet recorded. The lowest recorded is 0.2 millivolt. In twenty-one subjects eight have given values between 0.4 and 0.5 millivolt. We have not been able to correlate these values with age, sex, or other characteristics of the

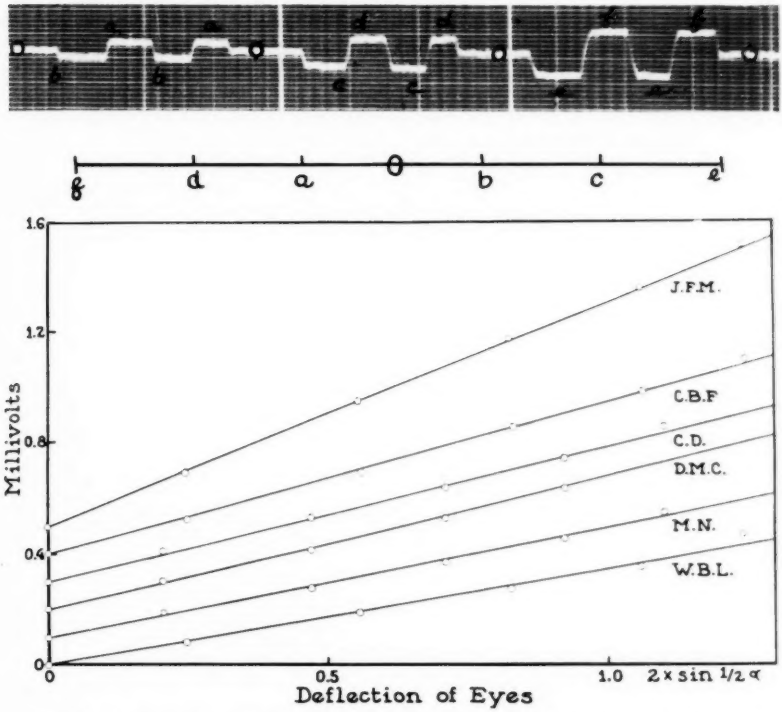

Fig. 1. Above, calibration record. Letters indicate points on wall at which the eyes were directed, as shown in middle diagram. Below, graphs of calibration data from six subjects. Each curve is plotted 0.1 m.V. above the one below to avoid confusion. All graphs actually begin at zero.

subject. Differences must depend upon differences in polarization of the eye ball, or differences in the effectiveness of the surrounding tissues in short-circuiting the potential. In any one individual, the potential is quite constant, successive values on one subject on different days being, for example, 0.19, 0.19, 0.23, 0.25, 0.16, 0.21, 0.18 millivolt.

We have used this method for various purposes which were secondary to our main objective. One of these is for recording eye movements in

reading. A sample record is shown in figure 3K. Each point of fixation on the line appears as a separate step in the record.

A second use for the method is for photographing nystagmus with eyes both closed and open. For this purpose, a Bárány chair was fitted with

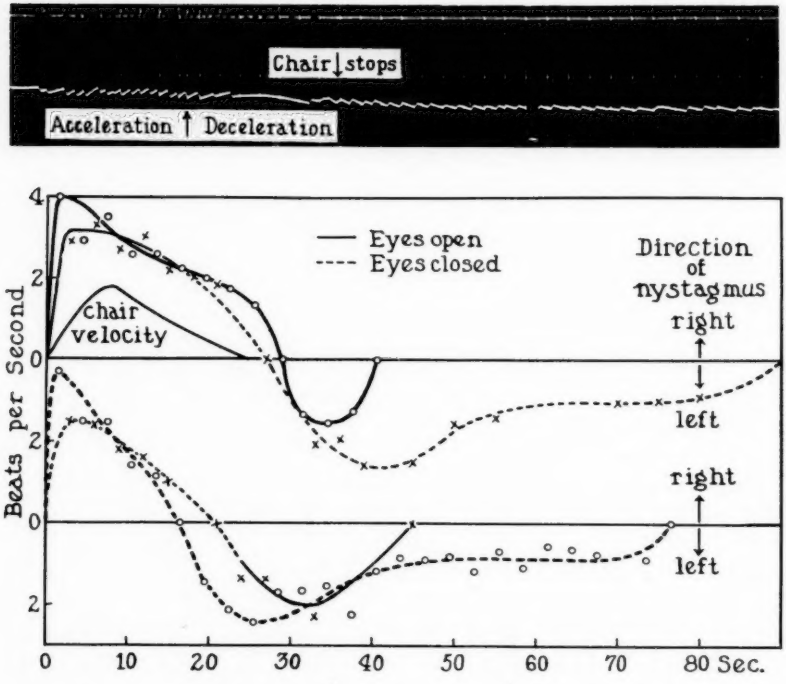

Fig. 2. Above, record of nystagmus due to rotation. Upper line is a record of a signal magnet, the circuit of which was closed 6 times in each revolution of the chair. Lower record is the shadow of the string recording movements of the eyes. Time in seconds. Extra signals made by passing hand through light when the accelerating weight hit the floor and when the chair stopped. Calibration deflection, 1 m.V. Eyes closed throughout. Below, graphs showing the frequency of nystagmus during and after rotation. Rotational nystagmus is plotted above the zero and the reversed after-nystagmus below the zero. Curve for chair velocity applies to lowest dotted graph only. Especially in the upper two graphs the spin was somewhat more prolonged. The reversal always occurs before the chair stops. After-nystagmus is much shorter when the eyes are open.

two brass rings underneath the seat which were connected by wires to the electrodes on the subject, and by brushes to the galvanometer. Thus records of eye movements could easily be obtained during rotation. A

sample record is shown in figure 2 (top), selected merely because it was unusually short. The chair was rotated by means of a falling weight which unwound a rope wound on a drum which was mounted on the axis of rotation of the chair, but over the head of the subject. The chair rotated to the right and the nystagmus during rotation was also to the right, as seen by the fact that the quick movements of the string are down in record, indicating that the eyes are moving to the right relative to the head. After the period of acceleration, there is an after-discharge which persists usually much longer than in the particular record shown, in spite of the fact that deceleration has begun. When the stimulus of deceleration becomes equal to the after-discharge, there is a period of quiescence in the string, and the subject experiences the familiar sensation that he is beginning to rotate in the other direction (subjective reversal) before the chair actually stops. The after-nystagmus begins at this time and continues for nearly a minute after the chair has actually stopped. The record shows clearly that the after-nystagmus is in the opposite direction to the rotational nystagmus, the quick strokes being up instead of down. If the eyes are open during this experiment, the rotational nystagmus is not noticeably affected, but the after-nystagmus is much abbreviated. It is similarly abbreviated if the eyes are closed during rotation but are open at the time of the subjective reversal. Graphs illustrating experiments of this sort are shown in figure 2. The result is confirmatory of the findings of Mowrer (1935) on pigeons.

Our original interest in this problem concerned the movements of the eyes which occur when the head is turned quickly around its vertical axis, from left to right or back again. If the eyes are open and one looks quickly from the right to the left a record similar to that in figure 3A is obtained. The upper record is the shadow of the short end of a long lever, the long arm of which is attached by a thread to the head of the subject. When the head is turned to the right it winds up the thread a little on the head and pulls the lever in such a direction as to give an upward deflection on the record. When the head turns to the right, the galvanometer string deflects downward, indicating that the eyes have moved to the right relative to the head which is turning to the right. Thus the eyes move to the right first and the head follows. This is readily confirmed by looking in a mirror. One can never see the head turn away since the eyes leave first. When one turns to look into a mirror on the other hand, one can see a slight movement of the head, indicating that it has just caught up with the eyes. The same pattern of movement usually takes place with the eyes shut, although the results are somewhat erratic (fig. 3B).

If the eyes are open and are fixated at an object in front and the head is rapidly wagged alternately to right and left, the eyes remain fixated.

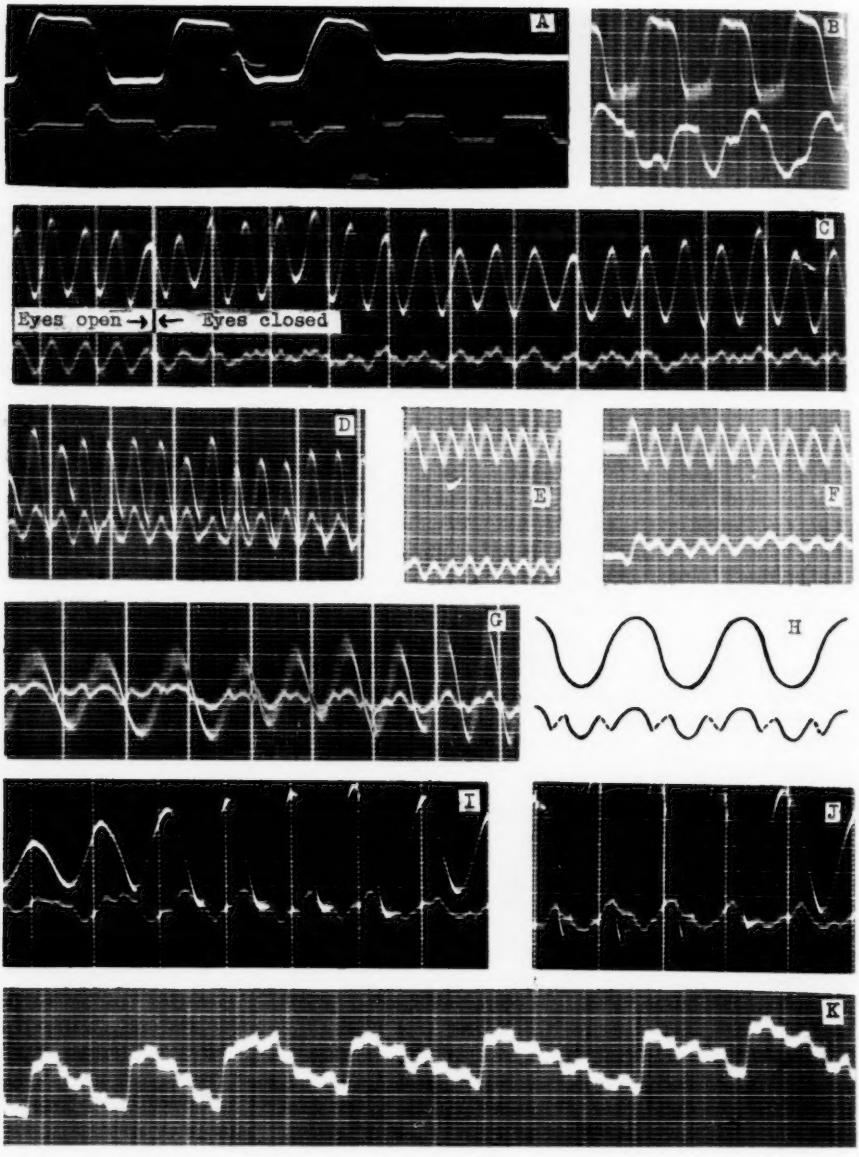

Fig. 3. Upper line in each record indicates movements of head to right (up) or to left (down). Lower line, string galvanometer, records movements of eyes to right (down) or to left (up). Time in $\frac{1}{5}$ second. Calibration potential of 1 m.V. is seen in A. A and B, looking to right and left alternately; C–J, wagging head; K, eye movements in reading. Each large upward deflection indicates movement of the eyes to the left to begin reading a new line.

This is involuntary and more or less inevitable. We were especially interested to discover what happened to the eyes when this same movement was carried out with the eyes shut. If the fixation was dependent upon visual reflexes it should disappear when the eyes were closed. To the extent that it was dependent upon labyrinthine reflexes it should persist. We did not consider at first what seems to be a third possibility, that the fixation could be so habitual that it would continue to occur in the dark from habit, i.e., as part of the pattern of movement.

If the eyes are open and fixated, a record shows that the movements of the head and those of the string are exactly parallel (fig. 3C). When the head is going right (up stroke of upper record) the eyes are going to the left relative to the head (up stroke of lower record) and the turning points are the same for both. A record of this sort serves as a good check on the accuracy of the recording methods. When the eyes were closed, in this same record, the eye movements were disturbed and the fixation was lost. Careful study of this record shows that what is happening is represented diagrammatically by figure 3H. In general, the eyes tend to stand still relative to the environment (fixation), but at intervals, represented by the dotted lines in the lower record, the eyes jump to catch up with the head. The movements of the eyes may then be described as fixation modified by nystagmoid movements. An actual record, which shows this type of movement more clearly, is found in figure 3G. For every maximum and minimum in the head record, there is a closely parallel maximum or minimum in the record of the string. The short, quick movements in between these maxima and minima are always in such a direction as to indicate that the eyes are moving in the same direction as the head, i.e., to catch up with it.

This nystagmoid-fixation type of movement is, however, only one of the three common patterns which are met with. Some individuals fixate just as well with the eyes closed as with the eyes open. Records E and F in figure 3 are cases in point, E being taken with the eyes open and F with the eyes shut. Careful inspection shows a close correspondence between the peaks of the two records. Furthermore, almost every individual can produce this type of record with the eyes shut if he is told to *imagine* that he is looking at some fixed point. Record D in figure 3 is a case of imaginary fixation of this sort. It was taken immediately after record C on the same subject with the eyes closed.

A third type of pattern may be called the "eyes-leading" pattern. It is really the same pattern which shows itself when one looks first to the right and then to the left (fig. 3A and 3B). As already mentioned, the eyes move first and then the head catches up. This also happens to some extent in the dark, and in many persons it happens when the head is wagged in rapid alternation with the eyes shut. Good examples are shown in

records I and J, figure 3. It will be seen that when the head record starts up the eye record starts down, and vice versa. Thus when the head is turning to the right, the eyes are turning to the right, relative to the head, or they are leading the head in the movement. Then the head catches up and the eyes again lead in the movement to the left. Many gradations are seen between these several different patterns. With the eyes closed, the eyes tend to fixate as before, probably because of labyrinthine reflexes. The labyrinth alone, however, does not seem to be able to maintain good fixation, because when the eyes are deviated more than a certain amount in the head they tend to carry out a quick overtaking movement as in nystagmus. Other habitual patterns of movement also tend to take control of the eye movements when the eyes are shut so that the result is not predictable for all individuals.

SUMMARY

Eye movements were recorded by a string galvanometer connected to electrodes on either side of the head lateral to the eyes. A calibration shows that the potential difference is proportional to the sine of the angle of deviation of the eyes. The potential developed varies from 0.2 to 0.8 millivolt, but is constant for any one individual. Records are presented of eye movements in reading and in rotation nystagmus. Graphic evidence is obtained of the great prolongation of the after-nystagmus resulting from keeping the eyes closed after rotation.

When the head is wagged rapidly right and left with the eyes closed, three types of movements are observed: 1, perfect fixation as when the eyes are open; 2, partial nystagmoid fixation in which quick overtaking movements of the eyes interrupt the fixation; 3, a pattern in which the eyes move in advance of the head as when one looks with eyes open from one side to another.

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CARBOHYDRATE METABOLISM OF HYPOPHYSECTOMIZED AND HYPOPHYSO-ADRENALECTOMIZED RATS[1]

E. L. COREY AND S. W. BRITTON

From the Physiological Laboratory of the University of Virginia Medical School

Received for publication August 4, 1936

The importance of the hypophysis in the regulation of carbohydrate metabolism has been specially recognized since the studies of Houssay and his associates (1931, 1932). The mechanism of this control is at present poorly understood. Krichesky (1936) found that the pancreatic islets of hypophysectomized rats increased in volume by about 63 per cent over that of his controls, and suggested that the hypophysis may exert a regulatory effect on the pancreas. Koster (1930) had previously found no islet changes, however, in the atrophic pancreas of the dog following pituitary removal.

Russel (1936) and others have found that all carbohydrate levels in the fully-fed, hypophysectomized rat remain within normal limits. The majority of observers (Smith *et al.*, 1936; Phillips and Robb, 1936; Fisher and Pencharz, 1936; Chaikoff *et al.*, 1935; and others) nevertheless agree in finding greatly lowered carbohydrate values in fasted hypophysectomized animals.

The present study was concerned particularly with the determination of carbohydrate values in unfasted hypophysectomized rats, in the hope that successive periodic analyses in a fairly large group of animals might yield data which would further elucidate the problem. A series of animals which were adrenalectomized at various times after hypophysectomy was also observed.

METHODS. Ninety-eight albino rats were successfully hypophysectomized by a modification of the method of Thompson (1932). Of these animals, 81 were males of mixed laboratory stock, and 17 were females of Wistar Institute strain.[2] The success or completeness of the operation was determined by *a*, gross examination in the region of the sella; *b*, the extent of testicular degeneration in male animals, and *c*, ovarian atrophy with cessation of estrus in females.[3] Cortico-adrenal atrophy (Smith,

[1] Grateful acknowledgment is made of aid received from the Rockefeller Foundation.

[2] Furnished through the generosity of Dr. M. J. Greenman.

[3] The cycles of all female animals were determined by the vaginal smear method of Long and Evans (1922) for at least 2 weeks previous to operation. One animal which showed an estrual type of smear 3 days following hypophysectomy was discarded.

1930; Cutuly, 1936) was considered as a corroborative sign of the success of pituitary removal. Animals were considered completely hypophysectomized only when so indicated by the above criteria, and data on other rats (7) were not utilized in this study.

Blood-sugar after hypophysectomy. A total of 172 determinations of the blood glucose of 98 rats was made from the time of operation until 100 days following hypophysectomy. The Folin-Malmros method was employed. An average of all values was made after a sufficiently representative number of determinations had been secured. The result is indicated in figure 1. Thus a slight fall in blood-sugar was evident during the 10-day period immediately following hypophysectomy; from this point onward, a fairly regular increase was seen, culminating in the highest levels observed between 30 and 40 days after operation. Thereafter the sugar values fell, on the average, to somewhat below the initial level (at 80 to 100 days after ablation of the gland). No positive statement can be made on the longest surviving animals, because of the small number of cases observed. The results show, therefore, a progressive increase in the blood glucose content of the hypophysectomized rat for about one month following pituitary removal, with a subsequent fall to normal or slightly lower in long-surviving animals.

Weight losses and mortality. It was thought that weight losses and mortality in the operated animals might be correlated with the observations on blood glucose (see figs. 1A and 1B). Loss in body weight was at first rapid, until 10 to 20 days after hypophysectomy. For the succeeding 10 days the animals maintained their weight somewhat better, but from 40 to 50 days after operation the average losses for the group were again accelerated. From 50 to 70 days following pituitary ablation all weights were, on the average, well maintained.

The survival period of all animals which died previous to experimental use was recorded, as shown in figure 1A. Of the 43 rats on which these mortality figures were based, 32 (or 47 per cent) died within 40 days of operation; 11 animals (26 per cent) survived from 40 to 60 days. The peak of the resulting curve coincided with the period of highest sugar content (30 to 40 days after operation).

It may be noted here that the findings of Aschner (1912), Pencharz *et al.* (1936) and others on the transitory polydipsia following complete hypophysectomy were confirmed, although a few cases only (4) were observed. Our data showed an average daily water intake of 18 cc. in control animals; a rapidly developing polydipsia occurred in the experimental rats, rising to an average intake of 50 cc. daily at the sixth day, and falling to normal at the eighth day after operation.

Tissue glycogen. Liver and muscle glycogen determinations were made on 32 animals, employing a modification of the method of Pflüger de-

scribed by Silvette and Britton (1932). These animals comprised those exhibiting the usual weight losses, lethargy and other conditions typical of hypophysectomy. Including all figures in the series, the average liver glycogen content at an average of 50 days after complete hypophysectomy was found to be 0.94 gram per cent. Thus liver glycogen cannot be considered to be critically reduced, although much below the normal control value of 2.44 grams. The average muscle glycogen content of the experimental animals was observed to be 0.43 gram, a level similar to that

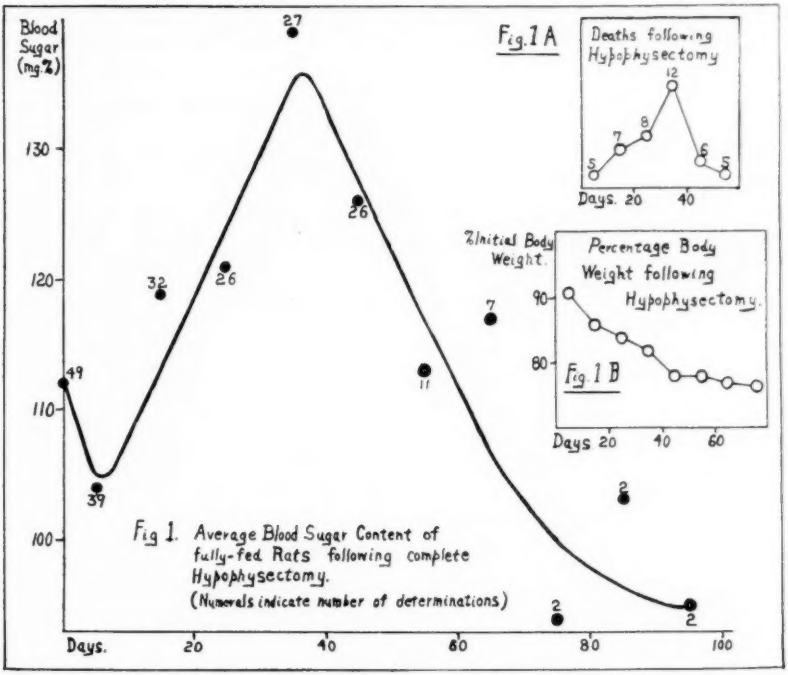

Fig. 1

of the controls (0.40 gram). These findings are in agreement with the results of Russel (1936) on fully-fed hypophysectomized rats.

Effects of adrenal removal on hypophysectomized animals. Britton and Silvette (1932) have for several years past pointed out the marked alterations in carbohydrate metabolism following adrenalectomy. Smith has called attention to the extensive atrophy of the adrenal cortex which follows excision of the hypophysis, and the present observations confirm his results. Swingle *et al.* (1936) have shown in a preliminary report the life-prolonging effects of hypophyseal and anterior-pituitary-like extracts

in adrenalectomized cats and dogs. The work of the Houssay school is now widely recognized. Furthermore, Long (1935) has demonstrated that total adrenalectomy produces strikingly similar results in pancreatic diabetes to those seen following hypophysectomy and suggested that the "hypophysis controls sugar formation from protein through the adrenal cortex." Hartman and Brownell (1934) have reported similar results. These observations, together with earlier results (Kitagawa, 1927; Wyman, 1928; Martin, 1932; Martin and Kroc, 1933; Corey and Britton, 1933, 1934) on the effect of adrenalectomy on estrus, strongly suggest an adreno-hypophyseal interrelationship. Thus a comparison of the effects of hy-

TABLE 1

A comparison of the effects of hypophysectomy and hypophyso-adrenalectomy on carbohydrate metabolism

RAT NUMBER	HYPOPHYSECTOMY		HYPOPHYSO-ADRENALECTOMY			
	Average blood-sugar over period of days indicated (mgm. per cent)		Days adrenal-ectomized	Blood-sugar (mgm. per cent)	Liver glycogen (gms. per cent)	Muscle glycogen (gms. per cent)
	days					
1	16	96	3	74	0.58	0.35
2	21	113	10	62	0.21	0.29
3	18	125	7	62	0.12	0.18
4	21	103	7	57	0.38	0.24
5	49	112	7	76	0.28	0.25
6	52	157	13	57	0.15	0.17
7	25	122	3	71	0.22	0.25
8	16	114	7	55	0.12	0.19
9	45	112	7	53	0.11	0.24
10	29	132	11	54	0.12	0.28
11	20	127	14	77	0.34	0.20
12	24	153	10	70	0.14	0.23
Average..	28	121	8	64	0.23	0.24

Average blood-sugar before hypophysectomy 104.

pophysectomy and adrenalectomy on carbohydrate metabolism in the rat was considered desirable.

Table 1 presents the results in summary. The average blood-sugar level in 12 animals which were successfully hypophysectomized rose as indicated from 104 to 121 mgm. per cent in 28 days. The animals were then adrenalectomized, and after an average interval of 8 days (the animals being sacrificed when symptoms of adrenal insufficiency appeared, 3 to 14 days after operation), blood-sugar and liver and muscle glycogen determinations were made. It will be noted from the table that blood glucose in these animals was reduced by almost 50 per cent during this post-adrenalectomy interval. Diminution in hepatic and muscle glyco-

gen levels was also very pronounced—much more than following hypophysectomy alone.

Discussion. In evaluating the results on blood-sugar following hypophysectomy, it appears that all the figures recorded might be considered within the normal variation for unfasted (absorptive-stage) rats. It is to be emphasized, however, that a general tendency toward an increased sugar content was revealed, beginning about 10 days after operation and continuing for 30 to 40 days. The slight fall in blood-sugar which was evident during a short period immediately following hypophysectomy might be ascribed to the after-effects of the operation itself, perhaps especially a temporary anorexia.

Apparently little pertinent information may be gathered from the weight-loss determinations and mortality data. The weight loss was at first rapid and progressive until about 45 days after operation; subsequently there was little change observed. There was no abrupt decrease in body weight coincident with the onset of blood-sugar reduction. The period of highest mortality rate was found to coincide with the highest sugar determinations recorded, rather than with an average low value.

Variations in liver glycogen content of hypophysectomized rats were notable. These may be referable to individual reaction to operation and the extent of post-operative anorexia resulting from irritation in the area of incision, as well as possible genetic differences.

Several of the liver and muscle glycogen values observed following hypophysectomy were as low as those observed after hypophyso-adrenalectomy. However, the majority of the hypophysectomized animals showed quite normal glycogen values coincident with every indication of complete pituitary ablation, as judged by the criteria described. The hypophyso-adrenalectomized series yielded, on the other hand, consistently low values in both liver and muscle glycogen, as well as in blood-sugar. Although carbohydrate depletion is known to follow the fasting of hypophysectomized animals, the anatomical alterations in the adrenal cortex (referred to above) together with the results obtained in the present study suggest that the adrenal cortex may be primarily concerned in the alterations in carbohydrate economy which are observed following injury to or ablation of the hypophysis.

SUMMARY

In a series of 98 completely hypophysectomized rats, the average blood-sugar content rose for 30 to 40 days following operation to approximately 25 per cent above average normal values. A subsequent fall to somewhat below the normal level occurred in long-surviving animals. These carbohydrate changes were apparently not correlated with nutritional or weight changes following hypophysectomy, or with the survival period.

Observations by other workers on transitory polydipsia following ablation of the hypophysis were confirmed.

Liver and muscle glycogen values in hypophysectomized (unfasted) rats varied widely, but were found to be on the average within normal limits.

Adrenalectomy following hypophysectomy resulted in rapid and pronounced diminutions in blood-sugar and liver and muscle glycogen levels.

Carbohydrate changes which are observed in the chronic condition following hypophysectomy may be in part referable to effects on the adrenal cortex.

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FURTHER OBSERVATIONS ON SODIUM CHLORIDE BALANCE IN THE ADRENALECTOMIZED OPOSSUM[1]

S. W. BRITTON AND H. SILVETTE[2]

From the Physiological Laboratory of the University of Virginia Medical School

Received for publication August 4, 1936

Blood and muscle tissues of the adrenalectomized opossum contain higher concentrations of sodium and chloride than those of normal animals, according to recent observations (Britton and Silvette, 1936; Silvette and Britton, 1936). The increased salt levels are apparently due to diminished renal excretion of sodium and chloride. Since these findings differ so paradoxically from those reported by ourselves and others after excision of the adrenal glands in dogs, cats, rats and other higher mammals (Baumann and Kurland, 1927; Harrop *et al.*, 1933; Loeb *et al.*, 1933; Silvette and Britton, 1933, 1935), it seemed desirable to consider the question more extensively and attempt to discover the mechanism of the phenomena involved. The chemical and operative procedures used have been previously described (Silvette and Britton, 1932, 1933, 1935).

RESULTS. *Fasting normal opossums.* Following two to three weeks of fasting, in which water was supplied *ad lib.*, the unoperated opossum maintained its normal serum sodium and chloride concentrations. The protocols below are illustrative:

Opossum 1, male 2/28/35, wt. 2.98 kilos; food withheld, water *ad lib.*, 19 days. 3/19/35, animal weak, wt. 1.38 kilos; sacrificed. Serum Na 333 mgm., Cl 388 mgm., sugar 82 mgm. per cent. Liver glycogen 0.15 per cent. Muscle glycogen 0.20, Na 0.110, Cl 0.057 and water 76.1 per cent.

Opossum 2, male, 3/12/35, wt. 1.42 kilos; food withheld, water *ad lib.*, 17 days. 3/29/35, animal weak, wt. 1.00 kilo; sacrificed. Serum Na 337 mgm., Cl 388 mgm., sugar 120 mgm. per cent. Liver glycogen 0.10 per cent. Muscle glycogen 0.13, Na 0.043, Cl 0.042 and water 77.8 per cent.

Apparently in this animal as well as higher forms the serum sodium and chloride levels are important enough to be preserved unchanged as long as possible under physiologically difficult conditions.

Fasting adrenalectomized opossums. Adrenalectomized opossums under fasting conditions with water allowed were found at the time of utilization,

[1] Grateful acknowledgment is made of aid received from the Rockefeller Foundation.

[2] E. R. Squibb and Sons Scientific Fellow in Physiology.

TABLE 1

Sodium, chloride and carbohydrate levels in opossums under different conditions

	SURVIVAL	SODIUM		CHLORIDE		MUSCLE WATER	SERUM SUGAR	GLYCOGEN	
		Serum	Muscle	Serum	Muscle			Liver	Muscle
Normal controls (18 cases)*									
	days	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*per cent*	*mgm. per cent*	*per cent*	*per cent*
Average		336	68	382	56	77.6	100	0.66	0.48
Adrenalectomized, no treatment (11 cases)*									
Average	6	360	80	411	60	72.7	69	0.13	0.28
A. Adrenalectomized, no food, water ad lib. (5 cases)									
Maximum	22	314	88	395	57	78.6	94	0.21	0.37
Minimum	4	232	12	320	31	76.6	52	0.03	0.10
Average	11	284	50	360	44	77.7	64	0.12	0.26
B. Adrenalectomized, no food or water (3 cases)									
Maximum	8	354		317		83.3	83	0.16	0.60
Minimum	2	318		363		78.1	49	0.13	0.38
Average	6	341		396		80.5	66	0.15	0.42
C. Adrenalectomized, dehydration (diarrhea) (9 cases)									
Maximum	14	309	88	414	67	79.4	93	0.35	0.69
Minimum	3	232	55	272	38	72.0	57	0.08	0.20
Average	10	272	73	361	49	76.6	66	0.14	0.35
D. Normal, lactating (3 cases)									
Maximum		320		368		80.2	153		
Minimum		297		357		76.7	144		
Average		310		364		78.1	148		
E. Adrenalectomized, lactating (3 cases)									
Maximum	33	264		345			81	0.40	0.44
Minimum	11	234		322			55	0.09	0.05
Average	22	252		336			69	0.20	0.21

* Averages from paper by Silvette and Britton, 1936.

when insufficiency symptoms had appeared, to show reduced serum and muscle sodium and chloride levels (table 1, A). The contrast of the results to those previously reported on normally-fed adrenalectomized opossums (in which serum sodium and chloride were definitely increased) led us to determine whether the withholding of food with free access to water might not have resulted in dilution of blood and extra-cellular fluid, and thus in an apparent lowering of the salt levels. Another series of animals was therefore deprived of both food and water after adrenal removal. The serum sodium and chloride concentrations at the time of utilization of these animals were found to be slightly increased (table 1, B).

Normal lactating opossums. A series of normal female opossums which were suckling young in the marsupium was studied. In these animals, which had been kept under laboratory conditions for one to three weeks, the serum sodium and chloride levels were found to be reduced. At the same time, muscle water levels were higher than those of comparable tissues from normal non-lactating females and normal males (table 1, D). The possibility that the diets used contained no more than the minimum needs of sodium for animals under no physiological necessity for increased salt was entertained. The salt requirements of lactating female opossums were obviously greater, therefore, than those of non-nursing animals.

Adrenalectomized lactating females. A small group of lactating females was adrenalectomized and their young allowed to nurse after the operation. In these cases serum sodium chloride levels were observed to have diminished markedly at the time insufficiency symptoms had set in (table 1, E).

Adrenalectomized opossums with dehydration (diarrhea). A group of non-lactating female opossums was adrenalectomized and subsequently fed almost wholly on cow's milk, a diet which readily produces a severe diarrhea even in normal animals. When insufficiency symptoms appeared the animals were used. Serum sodium and chloride levels were found to be considerably reduced, and the muscles showed evidences of dehydration (table 1, C). These results on opossums are in keeping with the well-known observation that in cases of severe diarrhea in man, serum sodium disappears to a relatively greater degree than serum chloride, due to excessive base loss *via* the intestine.

DISCUSSION. Whenever extrarenal excretion of sodium chloride becomes quantitatively important, as in lactating females and in animals suffering from profuse diarrhea, adrenalectomy in the opossum is followed by reductions in serum sodium and chloride concentration. The averages show marked decreases from the normal serum sodium levels, amounting to between 20 and 25 per cent (fig. 1). This is in contrast to increases which are observed in adrenalless opossums in which the insufficiency follows the usual uncomplicated course. Thus the opossum kidney, whether directly or indirectly influenced by the absence of a cortico-ad-

renal hormone, is able to hold back sodium and chloride excretion. A discussion of this matter has been put forward in a recent paper (Silvette and Britton, 1936). No hormonal regulation of other paths of salt loss, e.g., mammary glands and large intestine, is apparent from these experiments.

The reductions in serum sodium observed in fasted (but not thirsted) adrenalectomized opossums may also be explicable on a simple basis. While the salt intake is of course reduced to zero, the animals continuously excrete urine containing at least small quantities of salt. In this connection it may be observed that untreated opossums lived on the average six days after adrenal excision, while the group deprived of food but not of water survived and excreted endogenous salt for eleven days.

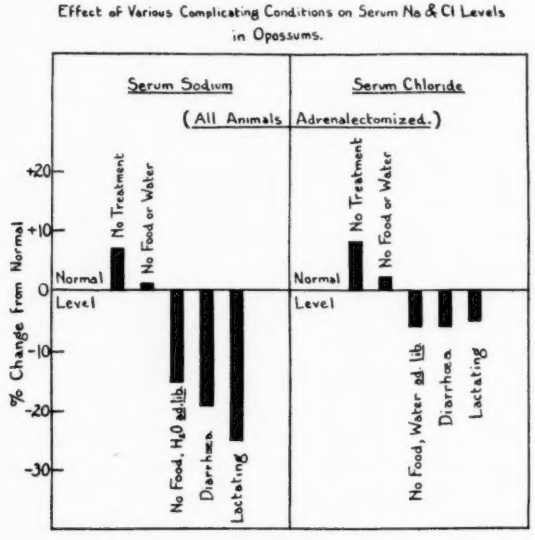

Fig. 1

Adrenalectomized opossums from which both food and water were withheld might be considered, for the short space of the six-day survival period, as being in salt equilibrium. Thus, while salt intake was again zero, salt (and urine) output were undoubtedly minimal. Concentrations of serum sodium and chloride were found to approximate those observed in animals in which the salt intake was normal and the urinary output reduced

Sodium and chloride concentrations in the opossum appear to be in a state of delicate balance as in higher mammalian types, for salt loss *via* kidneys, intestine or mammary glands leads, as in the cat and dog, to

depletion of serum and muscle sodium and chloride. On the other hand, reduction of urinary output of salt observed in uncomplicated adrenal insufficiency in the opossum leads to an increase in sodium chloride concentration throughout the body. It appears that serum sodium chloride levels, whether high or low, are merely reflections of increased or decreased renal or extrarenal excretion of salt, and not to redistribution of salt between blood serum and tissues.

It is to be emphasized, however, that following adrenalectomy in the opossum, no matter what additional complicating procedure may have been superimposed, serum sugar is reduced to levels below 70 mgm. per cent and liver glycogen to approximately 0.1 gram per 100 grams of tissue.

SUMMARY

Further observations on sodium and chloride levels in the opossum indicate a delicate salt balance even in this primitive form, when emergency conditions are imposed.

Opossums suffering from adrenal insufficiency (average survival period 6 days) show definite increases in serum sodium and chloride levels, in contrast to decreases observed in higher mammalian types under the same conditions. Long-continued fasting (18 days, water allowed) nevertheless produces little change in serum sodium and chlorides.

Adrenalectomized opossums from which food and water were withheld following operation survived an average of 6 days and showed normal or slightly increased serum sodium and chloride levels. Adrenalectomized animals from which food alone was withheld showed markedly diminished serum sodium levels and less marked depletions in serum chloride concentration. Opossums which during the course of adrenal insufficiency lost salt by extrarenal pathways, such as lactating females and animals suffering from diarrhea, showed similar chloride and even greater sodium reductions.

Regardless of any complicating influence on the course of adrenal insufficiency, adrenalectomized opossums at the time insufficiency symptoms were developed showed low serum sugar and liver and muscle glycogen levels.

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TONE IN THE MAMMALIAN VENTRICLE

VICTOR JOHNSON AND LOUIS N. KATZ

From the Cardiovascular Laboratory, Department of Physiology, Michael Reese Hospital, and the Department of Physiology, University of Chicago, Chicago, Ill.

Received for publication August 5, 1936

It has been repeatedly demonstrated that tone changes occur in the auricles and ventricles of cold blooded vertebrates. Because this is not true of the mammalian ventricle, various investigators have defined the term "tone" in different ways, which, as Meek (1927) points out, has introduced considerable confusion into this field. We believe, with Wiggers (1923) and Meek (1927) that the term should be employed specifically to designate "a sustained partial contraction, independent of systolic contractions, by virtue of which the muscle fibers resist distention during diastole more than they would because of their mere physical properties." Wiggers and Meek both conclude that no satisfactory evidence has yet been advanced for the existence of tone in this sense, or for variations of such tone under physiological conditions, in the mammalian ventricle.

Certain conditions must be rigorously fulfilled in any experimental demonstration of tone: first, attention must be paid not only to the length of the muscle fibers in the relaxed state, but also to the tension to which the fibers are exposed. The former (i.e., the initial volume, or I.V.) can be determined by measuring the diastolic volume just before the onset of systole, when the ventricular musculature approximates complete relaxation. The latter (i.e., the initial pressure, or I.P.) can be determined by measuring the pressure existing within the ventricles at this time. It is in reality the ratio of initial pressure to initial volume (I.P./I.V.) and not one or the other alone, which is significant. A convincing demonstration of a change in tone would require that I.P. and I.V. can and do change in opposite directions, or independently of one another.

Since it has been shown that the initial pressure in the right ventricle (R.I.P.) and that of the left ventricle (L.I.P.) do not always change in the same direction (Katz, Ralli, and Cheer, 1928; Brams and Katz, 1931) it becomes necessary to observe the initial pressures in both ventricles, and to show that *both* the L.I.P. and the R.I.P. may change in a direction opposite to the change in I.V.

Second, ideally, L.I.P., R.I.P., and I.V. should always be determined

when ordinary diastolic relaxation is complete, so that the I.P./I.V. changes which occur normally in the course of each cardiac cycle would be ruled out. It is not always possible to maintain a slow enough cardiac rate to insure complete diastolic relaxation, though usually this condition can be approximated. But because relaxation may not be complete, it is absolutely necessary that strictly corresponding or homologous points of the cardiac cycles be used for the determinations of I.P. and I.V., under varying experimental conditions. This can be done only by maintenance of a constant heart rate throughout any one experiment. If we look ahead to figure 2, the importance of this point becomes clear. In this figure, both the R.I.P. and the L.I.P. in the second beat (point *B*) are the same as in the first beat (point *A*). Yet the I.V. in the second beat is some 7 cc. greater than in the first. There has been a diminution in the ratio of pressures to volume which is obviously due to the fact that at the faster rate (first beat) the heart has not had time to relax as completely as at the slower rate, by the time the next systole begins. Points *A* and *B*, in other words, are not homologous points in these two cycles, and the change in ratio of pressure to volume which has occurred, does not represent a tone change.

Likewise, even if the heart rate is kept slow and constant, any decided alteration in the duration of systole or the phases of diastole would make a comparison of homologous points impossible. Even though the duration of each entire cycle remains constant, any change in the duration of any of these phases within the cycle would render the curves useless for analysis of tone changes, since the diastolic points compared would again not be homologous.

Failure to take these factors into account, as well as failure to employ adequate recording methods, make it impossible to consider as valid evidence published data purporting to demonstrate tone changes in the mammalian ventricle. For example, the work of Mansfield and Hecht (1933) may be criticized because their tracings were made by inadequate recording apparatus on a drum revolving too slowly to permit of analysis of the phase durations of the cycles. Also, they measured the pressure changes in only one ventricle. Finally it may be said that the hearts they used were in poor condition, having in some cases a stroke volume of only 5 cc. Similarly, we feel that while such studies as those of Van Liere, Crisler, and Hall (1934) might yield important data regarding cardiac dilatation, they cannot be interpreted in terms of tone changes, because it is unsafe to assume that initial intraventricular pressures will remain constant. They must actually be recorded at the same time that the diastolic volume changes are being observed.

It has been our aim in the experiments here reported, to take these significant factors into account. For this it was necessary to develop a

technique for simultaneous recording of the ventricular volume curves and the pressure curves of each of the two ventricles. Such curves offer new possibilities in the analysis of the dynamics of the cardiac cycle, although in the present report we confine ourselves primarily to the information they give concerning the problem of tone.

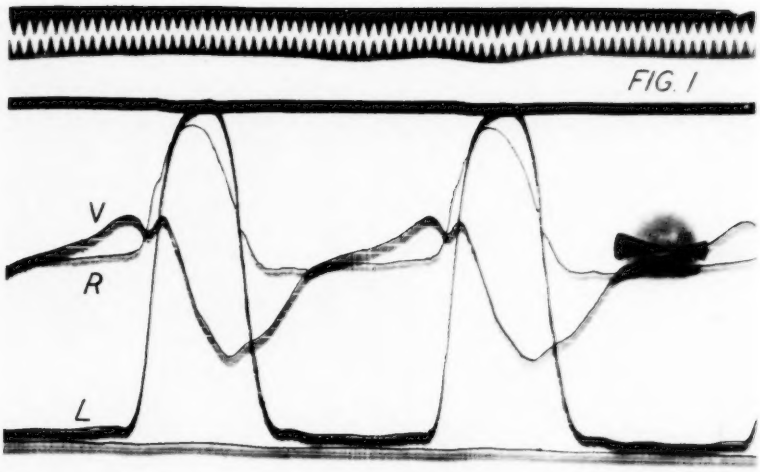
METHOD. Medium sized dogs anesthetized with sodium barbital intravenously, were used. The chest was opened widely, hemorrhage was controlled by cautery, artificial respiration at a constant rate instituted, and the pericardium opened. Fine copper wires were attached to the right auricle to permit driving the heart at a constant rate with intermittent break shocks delivered by a Lewis interruptor. In order to obtain a sufficiently slow rate it was usually necessary to depress the spontaneous rate of the sinus pacemaker by removing the stellate ganglia, and in addition, in some cases, by lightly tetanizing the peripheral end of the cut right vagus nerve, or by slowly infusing dilute mecholyl solution[1] intravenously (e.g., 0.25 cc. of a 1 to 5,000 solution, per minute).

Wiggers manometers were inserted into the two ventricles, the right via the azygos vein through the atrio-ventricular orifice, the left via the subclavian artery through the aortic orifice. The technique employed, including use of base lines and calibration of the manometers was similar to that described by Wiggers (1928). Parallax was avoided in recording the curves optically by the use of double slit lamps, as suggested by Katz and Baker (1924). The sensitivity of the manometers was greater than ordinarily used to facilitate detection of rather small changes in initial tension. A change of pressure of 10 mm. Hg caused a deflection on the right ventricular pressure curve of approximately 12 mm., and on the left, of 10 mm., on the average.

The ventricles were enclosed in a glass oncometer for volume recording. The oncometer was a modification (in shape) of that used by Wiggers and Katz (1922), and the method of optical registration was as described by them. Care was taken, of course, to insure a proper fit of the rubber membrane of the oncometer at the A-V groove, to avoid both leakage and constriction. A check of the system showed the accidental movements of the rubber diaphragm of the oncometer produced insignificant fluctuations in the volume curve. At the close of each experiment, after cessation of the heart beat, the oncometer was calibrated by injecting into the oncometer-capsule system, or removing from it, known volumes of air. No attempt was made to determine the absolute volume of the heart.

Records were taken, with artificial respiration temporarily arrested, at various times in the course of the experiment, and the following procedures were designed to alter circulatory conditions. The procedures employed were: *a*, modification of the resistance to ejection by the left ventricle, by varying degrees of stenosis of the lower thoracic aorta produced by means

[1] Kindly supplied by Merck and Company.



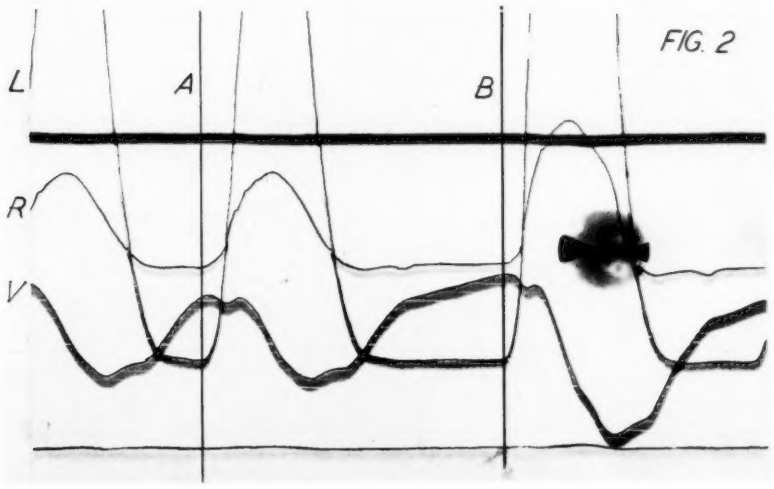


Fig. 1. Simultaneously recorded curves of ventricular volume, *V*, right intraventricular pressure, *R*, and left intraventricular pressure, *L*. Lower base line, for left intraventricular pressure and ventricular volume curves. Upper base line, for right intraventricular pressure curve. Time, each double vibration equals 0.02 second. The upper edge of each curve has been outlined with india ink in this and succeeding figures.

Fig. 2. Curves demonstrating the effect of heart rate changes upon the R.I.P./I.V. and L.I.P./I.V. ratios. At *B* the initial ventricular volume is greater than at *A*, although the initial pressures in each ventricle have not changed. This is not a tone decrease, because the duration of the diastole preceding *B* was longer than that preceding *A*. *A* and *B* are not corresponding or homologous points in the cardiac cycle.

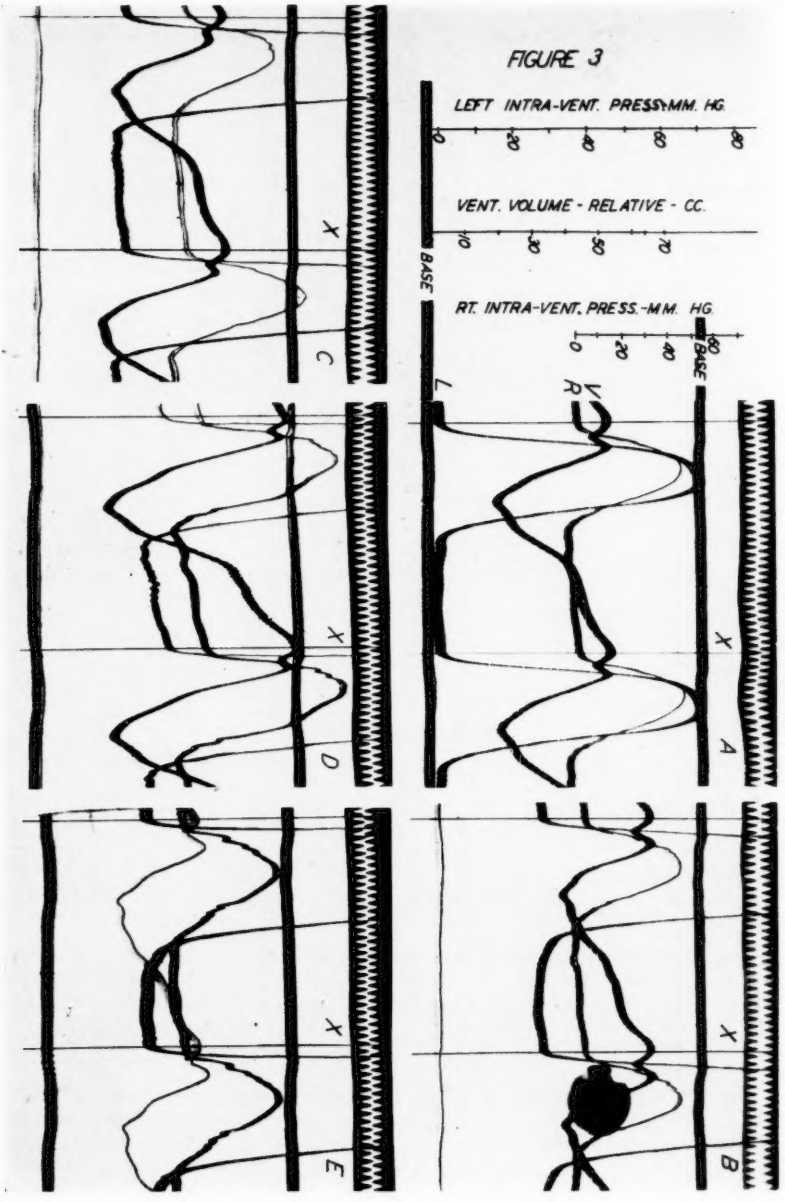


FIGURE 3
LEFT INTRA-VENT. PRESS.-MM. HG.
0
20
40
60
80
VENT. VOLUME - RELATIVE - CC.
10
30
50
70
BASE
RT. INTRA-VENT. PRESS.-MM. HG.
0
20
40
60
BASE
L
R
X
C
D
A
E
B

of an adjustable clamp (besides modifying peripheral resistance, this procedure would also modify the coronary flow, and therefore also, the physiological condition of the heart muscle); *b*, modification of the venous return to the right heart by intravenous saline infusion; *c*, in addition, spontaneous changes occurring in the failing heart at the close of an experiment often yielded valuable data.

The curves so obtained were analyzed for evidences of changes in the power of the heart, but attention was directed primarily to the R.I.P., the L.I.P., and the I.V. A vertical line (marked *X* on the tracings of figs. 3 to 6) was drawn just before the onset of systole, as indicated by the sudden rise of intraventricular pressure. All measurements of R.I.P., L.I.P., and I.V. were made on this line. The asynchrony of initiation of systole in the two chambers was not sufficient at this camera speed, to introduce significant errors. There was no parallax, and actual trial showed no phase displacement of the volume curve. Furthermore, in our analyses, attention was paid to changes which were definitely outside the experimental error involved in recording and measurement.

A second vertical line was drawn to mark the onset of the preceding cycle. The time interval between the two lines shows the duration of the cycle immediately preceding the point at which the measurements were made. In none of the experiments did this value vary by more than 0.01 second from one record to another in any single experiment.

Results. Typical results are shown in figures 1 to 6. Figure 1 shows two heart cycles in which all curves are recorded, and gives an idea of the cyclic changes in ventricular volume and in the pressures in the two ventricles. Analysis shows a degree of independent variation of events in the two chambers. Since the manometers are of nearly equal sensitivity, and the volume output of each ventricle is approximately the same, these curves can more readily be used to analyze the details of cardiodynamics than has been possible by combinations of any two curves alone.

Thus in figure 2, comparison of the second and third beats shows how heart rate changes affect not only the initial volume and pressures, but

Fig. 3. Effect of modifying the arterial resistance upon pressure-volume relationships, showing a tone increase. Symbols, time, and base lines as in figure 1. Discussion in text.

RECORD	TIME	PROCEDURE	L.I.P.	R.I.P.	I.V.
	minutes		*mm. Hg*	*mm. Hg*	*cc plus (X)*
A	0	Control	1	4	53
B	2	Clamping of aorta	26	5	63
C	5	Release of clamp on aorta	22	10	56
D	8	Clamping of aorta	33	17	77
E	17	Continued clamping of aorta	25	11	43

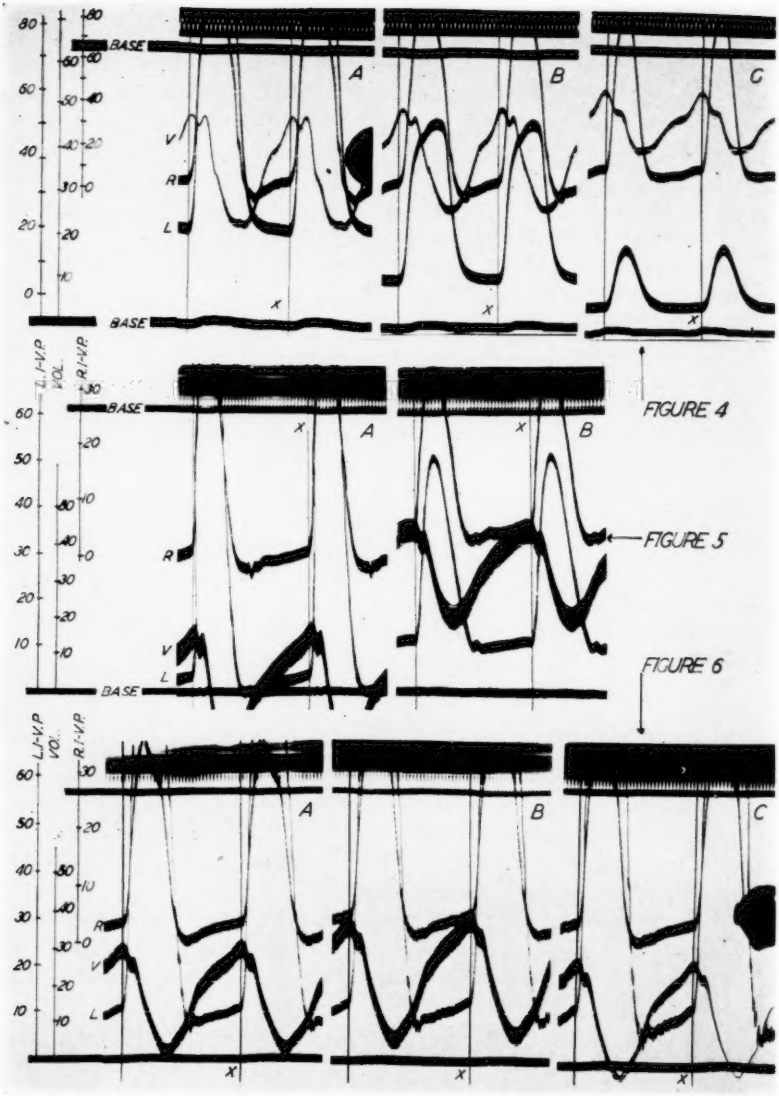


Fig. 4. Curves from a failing heart showing loss of tone. Symbols, time, and base lines as in figure 1. Discussion in text.

RECORD	TIME	L.I.P.	R.I.P.	I.V.
	minutes	*mm. Hg*	*mm. Hg*	*cc. plus (X)*
A	0	21	9	47
B	7	8	9	50
C	8	0	14	55

also sheds light upon factors affecting the stroke volume, for here the increased stroke volume at the slower rate is associated with an increase in I.V. without any appreciable modification of initial pressures in either ventricle. Further reference to this finding will be made later in another connection.

The ratios R.I.P./I.V. and L.I.P./I.V. are easily analyzed in figures 3 to 6. The five records in figure 3 show the effects of repeated tightening and release of the aortic clamp. When the aortic clamp was first tightened, between records A and B, the I.V. increased by 10 cc. (see table in legend of fig. 3). At the same time, however, there was a definite increase in the L.I.P., while the R.I.P. remained practically unchanged. Upon release of the aortic clamp (record C) the I.V. diminished again to approximately the control value. Simultaneously the L.I.P. fell somewhat, while the R.I.P. rose. This illustrates the fairly frequent observation that in the left and right ventricles the initial pressures may change in opposite directions. It clearly indicates the necessity for determining the initial pressures in *both* ventricles simultaneously, in studies on tone. If, for example, only the right intraventricular pressure and the ventricular volume curves had been recorded, we should have observed that the decrease in I.V. (from record B to record C) was associated with a rise in the (right) initial intraventricular pressure, and might erroneously conclude that the tone of the ventricles had increased. As it is, with our more complete data, we should have to concede that the diminution in I.V. here may have been due to the decrease in L.I.P., which is to say, the decrease in the distending force within the left ventricle.

In record D of figure 3, the peripheral resistance was again increased by clamping the aorta. Again the I.V. increased greatly. And simultane-

Fig. 5. Curves from another instance of heart failure showing no apparent tone change. Symbols, time, and base lines as in figure 1. Discussion in text.

RECORD	TIME	L.I.P.	R.I.P.	I.V.
	minutes	*mm. Hg*	*mm. Hg*	*cc. plus (X)*
A	0	4	3	18
B	2	11	8	46

Fig. 6. Effect of intravenous injection of 35 cc. isotonic saline solution, showing a small tone increase. Symbols, time, and base lines as in figure 1. Discussion in text.

RECORD	TIME	PROCEDURE	L.I.P.	R.I.P.	I.V.
	minutes		*mm. Hg*	*mm. Hg*	*cc. plus (X)*
A	0	Control	11	4	32
B	1	During injection	13	7	40
C	8	After injection	12	4	27

ously the initial pressures within each ventricle increased, as compared with the preceding tracing. That is, the increased ventricular size here must again be ascribed, most probably, to an increase in the distending pressures within the ventricles.

In records A to D nothing which can be interpreted as a tone change has yet occurred. However, there is definite evidence of an improved condition of the heart, presumably because of a better coronary circulation, effected by the clamping of the aorta. We see that as compared with record A, the gradient of pressure rise in record D is steeper, the systolic pressures attained are greater, and the stroke volume is markedly increased. With the cardiac musculature in this improved state, and continuing the aortic occlusion, we see evidence in record E of a ventricular tone greater than in the control record at the outset of the experiment. In record E the ventricles clamp down to a smaller size (by 10 cc.) despite increases in initial pressure in both ventricular chambers (24 mm. Hg in the left ventricle, 7 in the right).

Figure 4 shows tracings obtained toward the close of an experiment, just before the death of the animal. The changes in cardiac dynamics are clearly shown. In the 7 minutes elapsing between records A and B, the ventricles have dilated slightly. The R.I.P. has remained unchanged, but the L.I.P. fell by 13 mm. Hg. This is interpreted as a diminution in tone, accompanying heart failure. Later, (record C) the heart has dilated still further, and the L.I.P. is still further diminished. We cannot interpret this as a still further tone decrease, however, because in the meantime the R.I.P. has risen. Here again we see an example of changes in initial intraventricular pressures occurring in opposite directions in the two ventricles, and it may well be that the increased diastolic size in record C is entirely due to a greater distention from within the right ventricle, more than offsetting the diminished distending force acting in the left ventricle.

But even had the R.I.P. fallen with the L.I.P. it would still not be legitimate to interpret the changes in part C as a further tone decrease, because although the total duration of the whole cycle has not changed, the duration of diastole and diastasis has increased, and we should therefore not be comparing homologous points in the two cycles.

More frequent than the finding of a terminal tone loss was the observation of a simple dilatation, associated with increases in initial pressures. Figure 5 illustrates this. Here all three factors involved, R.I.P. L.I.P., and I.V. have increased as the heart fails.

Figure 6 shows the effect of increasing the venous return to the heart by injection of saline solution into the external jugular vein. During the injection (record B) the increased heart size was accompanied by increases in pressure in both ventricles, and later (record C) volume and pressures fell together. Even here there is evidence of a small tone increase. Com-

paring record C with record A, we see that there has been slight decrease in I.V. (5 cc.) with no appreciable change in either R.I.P. or L.I.P.

Discussion. These experiments demonstrate that the mammalian ventricles can display changes which satisfy rigid criteria of what constitutes tone or a change of tone, associated with other alterations in the dynamics of the heart. The infrequency with which apparent tone changes occurred deserves comment. In those instances where changes in the I.V. occurred along with changes in the R.I.P. in one direction, and L.I.P. in the opposite direction, it is not possible to state whether the tone changed or not. To analyze such situations we should have to know what volume changes occurred in *each* ventricle, which cannot be determined at present, or else, the relative effectiveness of the changes in distending pressures within each of the two ventricles in producing volume changes of the combined ventricles in the living, beating heart.

It is conceivable that even when initial pressures and initial volume change in the same direction, the curves of the ratios R.I.P./I.V. and L.I.P./I.V., plotted for a range of pressures, may be different under different physiological conditions, and might therefore disclose tone changes. Unfortunately, data of this sort are not available at present. At any rate, the possibility exists of tone changes being more frequent than were disclosed by application of the rigid criteria employed in our experiments.

Furthermore, it is recognized that the animals were not normal, and that the experimental method which must necessarily be employed at present, in making tone observations, might very well destroy, to a large extent, the very phenomenon which is being studied. The performance of pneumothorax, the exposure of the heart and trauma to it attendant upon inserting the cannulae, the partial denervation, and the use of artificial respiration must all tend to render the heart abnormal. There must also be some interference with normal valve action, produced by passing the cannulae into the ventricles. The left cannula must have produced some narrowing of the aortic orifice. However, the work of Katz, Ralli and Cheer (1928) indicates that dynamic disturbances occur only when the stenosis is very marked. Similarly, the narrowing of the tricuspid orifice produced by the right cannula was probably not significant, as Katz and Siegel (1929) have shown that only a marked A-V stenosis will cause dynamic disturbances.

At any rate, our observations suggest that in the normal, intact, unanesthetized animal, ventricular tone changes may occur. Whether these are more or less frequent than under our experimental conditions can only be demonstrated by further work.

The possible adaptive significance of tone changes is not entirely clear. In the few instances of tone changes we observed, an increase was associated with an improved condition of the ventricles, and a decrease with

failure of the heart, as evidenced by such indices of cardiac performance as the slope of the pressure curves, the intraventricular systolic pressures, and the slope and magnitude of the volume curves during systole. It is conceivable that a loss of tone, for example, might increase the capacity of a weakened heart to eject blood, through the operation of the Starling effect. At a given initial intraventricular pressure, an increase in diastolic fiber length through a diminution in tone, would tend to increase the subsequent systolic ejection, provided fiber length rather than initial tension per se, determines the Starling effect. This relationship has been shown to hold for the turtle (Katz, 1928). Evidence supporting this concept in the mammal is to be had from comparison of records A and E of figure 3. In record A, where tone is less, the fiber length (I.V.) is great, while the tension (R.I.P. and L.I.P.) is low. Yet the stroke volume is appreciably greater than in record E, where tone is greater. We have already referred to a similar relationship between fiber length, initial tension, and stroke volume in discussing figure 2, although in that instance no tone change was involved. Wiggers (1928) states that "there is at least more than a suspicion that changes in initial length, rather than initial tension, fundamentally determine the vigor of the response." He arrives at this tentative conclusion indirectly, from analysis of the dynamics of artificially induced premature ventricular contractions (Wiggers, 1925) and of the ventricles under the influence of digitalis (Wiggers and Stimson, 1927). In none of these experiments was it possible to make as complete an analysis of the factors involved, as in our experiments, with the three simultaneously recorded curves.

Of course any such possible beneficial action of decreased tone in the failing heart may become a detrimental factor when the ventricles are distended to the point where the Starling effect no longer operates.

On the other hand, an increase in tone occurring when the normal heart is put to a sudden strain, such as an increased resistance to ejection, may serve to supplement the action of the pericardium in preventing overdistention of the heart.

SUMMARY AND CONCLUSION

The term "tone" should be employed, in the case of the mammalian ventricle, only when referring to the relationships between fiber length, or initial ventricular volume, and the distending pressures in *each* ventricle, determined simultaneously for comparable points, under controlled conditions, especially as regards heart rate and duration of diastole.

In a few instances of occlusion of the lower thoracic aorta, with a resultant elevation of the aortic pressure and an improved coronary circulation, the left and right initial intraventricular pressures of dogs rose at the

same time that the initial volume fell. This is interpreted as an increase in ventricular tone.

The reverse change, that of a decreased tone, indicated by an increased ventricular volume despite a decrease in the distending pressures in the two ventricles, was observed terminally in the failing heart, in a few instances.

In most cases, occlusion or release of the aorta produced volume changes and distending pressure changes in the same direction. This likewise occurred in the few cases of saline injection. Such changes in cardiac volume cannot be interpreted at present as tone changes.

It is suggested that tone changes may be of some significance as an adaptation of the heart in failure, whereby a weakened heart with a reduction in tone may be enabled to eject more blood than it would were its tone undiminished. Incidental to this, evidence was presented which indicates that initial fiber length rather than initial intraventricular tension, determines the force of the heart beat. On the other hand, increase in tone of the normal heart when there is an increased resistance to ejection, may help to prevent overdistention.

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THE ALLEGED VALIDITY OF CORONARY SINUS OUTFLOW AS A CRITERION OF CORONARY REACTIONS[1]

J. RAYMOND JOHNSON AND CARL J. WIGGERS

From the Department of Physiology, Western Reserve University School of Medicine, Cleveland, Ohio

Received for publication August 7, 1936

The introduction by Morawitz and Zahn (16) of a special cannula, by means of which the coronary sinus can conveniently be drained to the exterior and the venous outflow measured, seemed to supply a simple method for studying coronary bloodflow in intact animals and heart-lung preparations. While the original belief that the entire return-flow is thus measured has been shown to be wrong, nearly all investigators are agreed that about 60 per cent of the total flow is consistently captured under greatly varying conditions of the flow including alterations in rate and force of heart beat. (For references and recent review *cf.* Wiggers, 18). Consequently, with the single precaution of keeping mean arterial pressure constant or taking its variations into account, the method continues to be extensively used for testing the effects of drugs and of nerve stimulation upon the caliber of coronary vessels. The profound importance of conclusions based upon such studies makes it imperative that the method be validated from many angles, or else that its limitations be defined more clearly.

The proposition that any change in total coronary flow will be reflected proportionally in the coronary sinus outflow has been questioned on both anatomical and experimental bases. In discussions before the American Physiological Society, one of us (W) has repeatedly directed attention to the fact that in perfused quiescent hearts of cats and dogs recently dead, the relative flows from the sinus (or stoppered atrium) and right ventricle show great variations, the ratios ranging from 1:1.3 to 1:4.8 in favor of Thebesian ventricular flow. Similar results were reported by Wearn (17) for human hearts. Apparently the anatomical resistance is generally less through vessels emptying into the right ventricular cavity than into the coronary sinus. If therefore a constant 60 per cent partition in favor of the sinus occurs in beating hearts, other physical factors must operate. Experimental data indicate that the three coronary branches do not contribute equally to the sinus flow. Anrep and King (4) found by tempo-

[1] The expenses of this investigation were defrayed from a grant by the Ella Sachs Plotz Foundation.

rary occlusion of individual branches that 70 per cent of the flow from the left coronary and 40 per cent from the right coronary artery drains into the common sinus. Katz, Weinstein and Jochim (15) found in fibrillating hearts that the right coronary may empty almost entirely through Thebesian vessels, the left circumflex ramus predominantly through these channels, and the left anterior descendens ramus almost equally through Thebesian vessels and the sinus. However many variations were found in different hearts. They properly concluded that unless vasomotor responses affect the entire coronary system equally, changes in coronary sinus outflow could not gauge the degree and perhaps not even the direction of change. Further, Katz, Jochim and Bohning (14) found that when the cardiac vigor is altered but the rate remains constant, the sinus outflow may vary in a direction opposite to and at times may exceed coronary inflow, indicating that the Thebesian vessels can serve as a portal of entry as well as of exit. If this is true, changes in sinus outflow cannot even be trusted to give evidence in regard to vasomotor changes in the tributaries that do drain into this common venous channel.

In public discussions as to probable causes, Katz has voiced the opinion that the effects are due predominantly to alterations in the dynamics of the right heart, changes in diastolic pressure being particularly stressed. We have long had similar ideas and as our investigations proceeded they crystallized into the following working hypothesis:

As pictured in figure 1, the blood after passing into the venous system—denoted schematically as a single vessel, *V*—can empty by numerous channels into the right ventricle as well as by the sinus and accessory veins into the right atrium. If there is no pressure in these chambers, a greater volume flows into the right ventricle, owing to a lower anatomical resistance. As in the case of other veins emptying into the heart, the natural efflux is determined no less by the rising and falling pressures in these chambers than by the pressures in the veins. This is well illustrated by the filling and collapse of the jugular veins. The approximate order of magnitude of the right atrial and right ventricular pressures is fairly well known and can be schematically indicated by the solid curves in figure 1. Since the pressures developed within the coronary veins remained unknown, we attempted to determine their magnitude and form in various branches and near the mouth of the vessel[2] but owing to technical difficulties imposed by the collapsibility of their thin walls and by the moving heart, we hesitated to accept any of the many variable records obtained. We have, for theoretical discussion purposes, selected the form and magnitude indicated by the dotted curves, *X*, of figure 1. It will be observed that it contains an atrial wave fairly comparable to that in the atrial

[2] We are indebted to Dr. Donald E. Gregg for his help in overcoming the technical difficulties involved in such registrations.

cavity and a systolic ventricular wave which rises to a peak of about 18 mm. Hg during initial phases of isometric contraction and after a sharp fall is maintained as a slightly rising plateau during ventricular ejection; after which it rapidly falls. Assuming such curves, the coronary venous pressure is at all times in excess of right atrial pressure, Y, but only markedly so during ventricular systole, consequently a pronounced flow might be anticipated only during systole. If however the flow were led to the

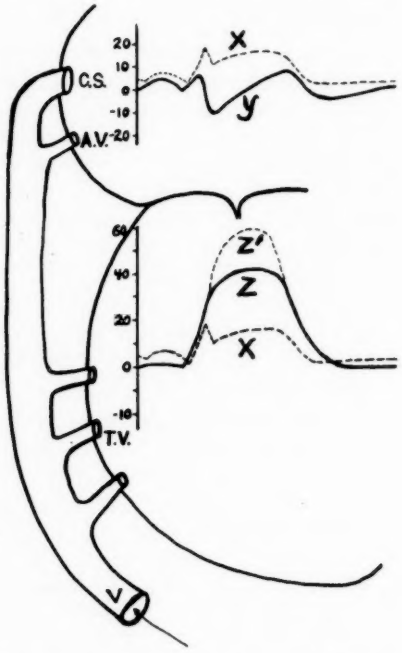

Fig. 1. Diagram illustrating the avenues for return flow from coronary veins and the theoretical pressure differences between intravenous pressures (curve X), atrial pressures (curve Y), and right intraventricular pressures (curves Z and Z'). Discussion in text.

exterior, against the constant atmospheric pressure (zero) this might be preceded by another onflow during atrial systole as found by Anrep *et al.* (3).

Since right ventricular pressure, curve Z, is markedly in excess of venous pressure, flow into the right ventricle would be stopped during the major portion of systole; indeed unless the communications are protected by valve-like structures, a backflow into the veins would be anticipated.

It is logical to suppose further that the blood thus prevented from entering the ventricle (or actually flowing back from the ventricle) may be directed into the coronary sinus. By such a dynamic scheme we could account for the proportionately greater drainage from the sinus in beating hearts (60 per cent) as compared with quiescent hearts (20 to 45 per cent).

A little reflection will show that an increase in the number of systoles per minute (systole-time) should increase the minute flow into the atrium from the sinus and accessory vein, provided coronary venous pressure remains the same and inflow into the venous channels, V, and right atrial pressure are unaltered. If no backflow occurs, variations in right ventricular pressure would be without effect provided the pressure height exceeds that in the veins. If however such entry takes place, the sinus flow should augment and almost in proportion to any increase in systolic pressure maximum, Z, when the systole-time remains constant. Since this is determined by the initial tension during diastole, our views are probably not essentially different from and are at least complementary to those of Katz and his associates.

It was the purpose of this investigation to determine experimentally whether there is any foundation for such a conception and if so whether it necessitates reinterpretation of sinus flow changes following use of drugs or nerve stimulation. In brief, we recorded the phasic changes in coronary sinus flow optically while right ventricular systolic pressure was increased by graded compression of the pulmonary artery, attention being given to maintenance of a constant heart rate and constant aortic pressure also optically recorded.

PROCEDURES AND APPARATUS. Dogs, anesthetized with morphine and sodium amytal[3] were used. The chest was opened with artificial respiration going; the pericardium was removed and apparatus connected for recording simultaneously aortic and right ventricular pressures together with phasic changes in coronary sinus flow.

Aortic and right ventricular pressure variations were recorded by use of Wiggers' optical manometers, calibrated under static conditions and inserted according to the technique employed in this laboratory. For recording coronary sinus flow, an adaptation of Frank's differential manometer (12) was used, our essential contribution consisting in devising a technique for its employment in registering coronary flow. Since we found in initial experiments that draining of the coronary sinus externally for only 6 or 8 beats abstracts enough blood to affect the aortic pressure, we devised a method by which the blood is returned at once to the superior vena cava. This also has the obvious advantage that flow occurs against a normally varying venous pressure and not against atmospheric zero

[3] We are indebted to Eli Lilly and Company, Indianapolis, for their generosity in furnishing a supply of this drug for experimental purposes.

pressure. A plan of the whole system is shown diagrammatically in figure 2. Blood drained from a coronary sinus cannula, *A*, flows through a rubber tube, *B*, and metal T-tube, *C*, thence through cannula, *D*, back to the vena cava. The internal diameters of all tubes are nearly equal to that of the coronary sinus opening. A second T-tube, *K*, allows drainage to the exterior when the tube to *D* is clamped. It proves useful in occasional checks of mean volume flows. Into the side-tube of the metal unit, *C*, is screwed the divided cannula, *E*, leading by lead tubes, *F* and *G*,

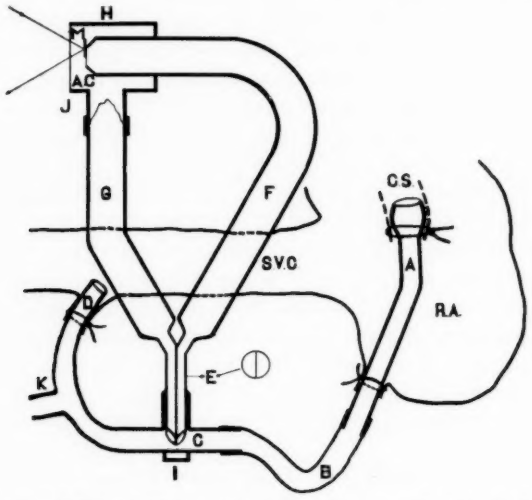

Fig. 2. Scheme of Frank differential manometer as applied to study of velocity changes in coronary sinus flow. *RA*, right atrium; *CS*, coronary sinus; *A*, coronary cannula; *D*, return cannula to superior vena cava (*SVC*).

Dimensions: Lead tubes *F* and *G*, 150 mm. x 9 mm.; tubes *E*, 30 mm. x 6.5 mm.^{2}; *Y* connections to tube *E*, 35 mm. x 6.4 mm.^{2}; cannula *A*, 100 mm. x 3.5 or 4.5 mm. Remainder of external circuit 235 to 285 mm. x 4.5–5 mm. Entire external circuit length 350–400 mm. Vibration frequency of entire system, 75 per second. (Second millimeter measurements refer to internal diameters; mm.^{2} to internal cross-section areas in each case.)

to the differential Frank manometer, *H*. A small removable screw-plug, *J*, at the bottom permits flushing of the tubes, *F* and *G*, from side stopcocks at their tops, not shown in the diagram.

To avoid such narrowing of the lumen as is introduced when the Morawitz cannula is employed, we used a simple tube with a short lip and appropriate curvature and tied this securely into the mouth of the coronary sinus. We were surprised to discover how easily a ligature can be placed around a cannula near the mouth of the sinus by a needle provided it is

started at the bottom of a constant depression on the posterior atrial wall which leads directly to one margin of the coronary sinus. We have called this the coronary fossa. Post-mortem injections were of course made after each experiment to verify the placement and tightness of the cannula.

The differential manometer of Frank (12) employing the principle of Pitot's tubes needs no detailed description. Briefly, the opening of the divided cannula pointing against the current transmitted pressure by tube, *F*, to the back surface of the rubber covering a small segment capsule; the cannula orifice pointing in the direction of the current, transmitted pressure through tube, *G*, to an anterior chamber, *AC*, and thus to the front of the rubber.

The movement of the rubber diaphragm is determined by the pressure difference from moment to moment which represents the velocity of flow. The device is calibrated by allowing fluid to flow at different rates through the system at the termination of an experiment.

Light is projected through a window in the anterior chamber upon a small mirror, *M*, pivoting upon the segment side of the capsule. To do so blood must be prevented from reaching this chamber. This is accomplished by introducing a lax miniature finger cot, *J*, made from rubber cement, over the end of a glass tube and subsequently vulcanized in sulphur chloride vapor. All parts of the apparatus are rigid but can be disjointed for thorough cleaning. The apparatus has numerous contrivances that facilitated filling, flushing and calibration and provided for a small flow of heparinized Locke's solution through the tubes when records were not being taken. These details are omitted in both our description and diagram.

The attachment of the apparatus so as to constitute a complete and uninterrupted coronary flow was accomplished in the following steps: 1. The coronary cannula connected to a closed rubber tube was first inserted into the atrial cavity and held by a purse-string suture not drawn too tightly or permanently.

2. A short cannula, also closed by a rubber tube was inserted into the right costo-cervical branch of the superior vena cava so that its orifice protruded into the lumen of the superior vena cava, as shown in the diagram.

3. The blood of the whole animal was rendered safely non-coagulable by one of three procedures *viz.*, a, use of 150 units of heparin per kilo; b, use of 75 units of heparin plus 0.08 gram chlorazol-fast pink, each per kilo, or c, use of 75 units of heparin per kilo, following a preceding fractional defibrination of about one-half the estimated blood volume of the animal.

4. The tubes of cannulae *A* and *B* were then connected and shortly after establishment of such a circuit, the tip of the coronary cannula was

introduced into the coronary sinus, a stitch was taken around it and tied. The coronary sinus flow was therefore not interrupted.

5. After alignment of the animal to the optical apparatus, etc., two operators quickly switched the connections through the unit *C*, causing only a momentary interruption of coronary sinus flow.

The other technical procedures included placing a screw-clamp around the descending thoracic aorta for the purpose a, of bringing arterial pressures up to normal values by a slight constriction, and b, of compensating for any fall of pressure following compression of the pulmonary artery, thereby maintaining a constant pressure head. Such graded pulmonary compression was accomplished by placing a ligature around the pulmonary artery and tightening the loop by a screw arrangement as described by Fineberg and Wiggers (11).

Since the flow was not recorded at the mouth of the coronary orifice but at a distance of 15 cm. (more or less), the delay in transmission needed to be determined experimentally for each length of tube used. This was generally of the order of 0.04 second. However as one learns to read the curves, no question as regards homonymous events need arise, even without such determinations.

Characteristics of normal flow curves. Figure 3A is a characteristic curve showing the velocity changes in sinus flow when the blood is led back into the superior vena cava. The vertical lines indicate the lag with respect to the aortic and right ventricular pressure curves. In contrast to records obtained by Anrep *et al.* (3) who led the blood to the exterior, only one definite wave usually appears during systole. It begins with onset of systole, *a*, and usually reaches its peak at or about the end of systole, *b*, and then drops off rapidly to a zero flow level. Curves from different experiments show considerable variations in contour (*i.e.*, suddenness of onset, steepness of slope, rounded or peaked character of summit, etc.) probably due to different dynamic conditions of the circulation at the time. In general the curves conform to the above description. Positive waves during atrial systole were never recorded but occasionally a negative presystolic wave deeper than that shown in the accompanying illustration was present. We are unable to convince ourselves that this represents an actual backflow and may well be due to unconquered mechanical movements of the cannula tip. The results accord with our theoretical analysis. The conclusion is reached that also in the intact heart the coronary sinus empties only during systole with the velocity of outflow increasing nearly to the end of systole. The diastolic flow is zero or insignificant.

By calibrating the apparatus, we can express the velocity of flow in cubic centimeters per minute. Such calibrations added on the records of figure 3 show that the curves do not have a linear proportionality; the sensitivity increases the higher the curves rise. To obtain correct velocities such curves need to be reconstructed (Broemser, 6). Since the deflec-

tion of the light beam is proportional to the square of the volume flow, it is possible by extracting the square root at consecutive points to de-

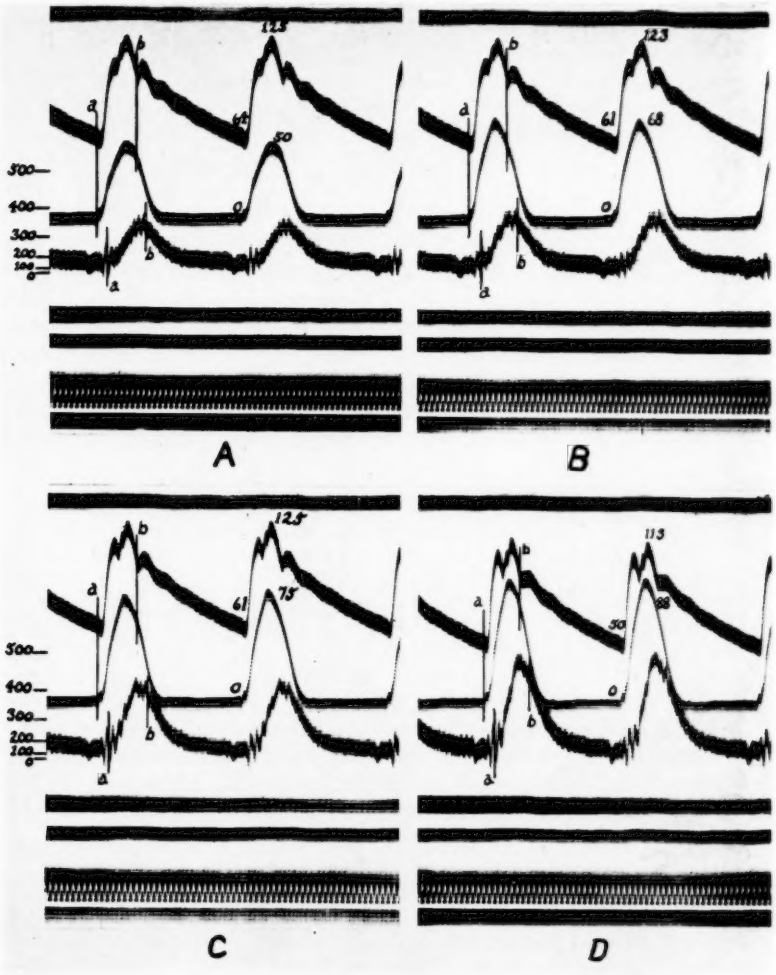

Fig. 3. Four sections of records showing effects of progressive increase in right ventricular pressure upon coronary sinus flow. In each curve: upper, aortic pressure; middle, right ventricular pressure; lower, coronary sinus flow. In addition, three base lines for these respective curves, time in 0.02 second, and calibration of coronary sinus velocity in cubic centimeters per second are shown. Discussion in text.

termine the volume flow either for a whole beat or for systole and diastole individually. By multiplying by the heart rate, the minute volume can

be determined. Such calculation of five experiments with pressures of 120/70, 125/64, 85/72, 113/84, 97/68 respectively gave flows of 58, 71, 32, 45 and 28 cubic centimeters per minute or 60, 71, 30, 35, 31 cc. per 100 grams heart muscle per minute. In these the systolic/diastolic distribution quotients were 1.92, 0.85, 1.05, 0.67 and 0.93 respectively. The magnitudes of flow agree sufficiently with those of previous investigators to serve as a check of the method and employment of roughly comparable conditions.

Effects of raising right ventricular pressure. The four sections of curves in figure 3 show the effects of progressive compression of the pulmonary

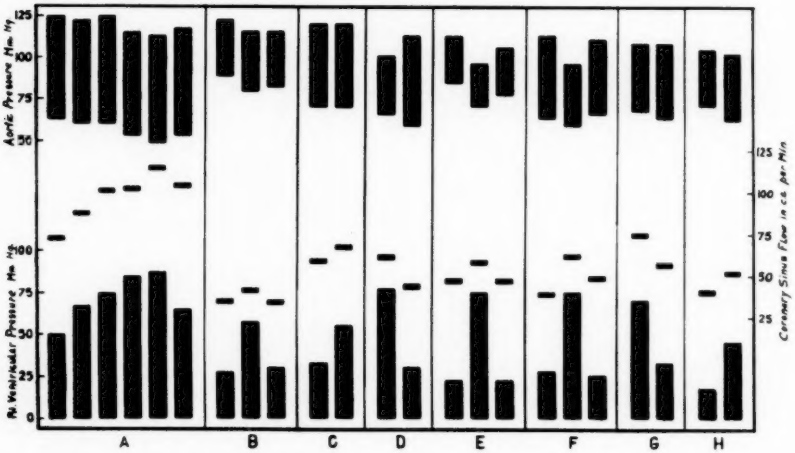

Fig. 4. Graphs showing variations in aortic pressure (upper blocks), coronary sinus flow in cubic centimeters per minute (middle lines) and variations in right ventricular pressures (lower blocks) following pulmonary arterial compression of different degrees in 8 dogs. Note that coronary sinus flow increases and decreases with right ventricular pressure maximum, sometimes regardless of slight changes in aortic pressure in opposite direction.

artery and in all but the last the aortic pressures were virtually unchanged. In this the effects were such as to cause a decrease in coronary sinus flow. With each increase in right ventricular pressure maximum, the steepness and magnitude of the systolic wave increase. Numerically expressed, the maximum velocity increased from about a 300 to a 460 cc. per minute level and the volume flow per cycle from 0.62 to 0.93 cc. or roughly 50 per cent. Since the heart accelerated slightly, the minute flow increased from 71.3 to 113.5 cc. per minute or about 60 per cent. The changes in aortic and right ventricular pressures in comparison to changes in coronary sinus flow obtained in a number of other experiments are graphically sum-

marized in figure 4. Since aortic pressures were in no case increased but always changed in a negative direction and since changes in heart rate never exceeded ±8 per cent, these results prove definitely that elevation of right ventricular pressure is associated with a proportional rise of coronary sinus outflow.

Discussion. To account for such relationship between systolic right ventricular pressure and coronary sinus flow, several factors must be considered, *viz.*, 1, a more vigorous compression of veins and venules, and 2, decreased drainage into the right ventricle with possible systolic regurgitation. The first seems to us an improbable factor for any such action would probably be cancelled by the greater resistance to flow of blood from arteries to veins, and moreover these influences would not affect tributaries from the left ventricle whose action—to judge from aortic curves—probably remained normal. Since, on the other hand, the gradients of the sinus flow curves change so definitely with those of ventricular pressure and since the effects are still present when aortic systolic pressure falls slightly (figs. 3D and 4 B, E, F), it is difficult to escape the conclusion that the augmented systolic flow from the sinus is due either to decreased Thebesian drainage into the right ventricle or perhaps even to a reversed flow.

We may now review the experimental evidence which has been interpreted as proving that the coronary sinus consistently drains approximately 60 per cent of the total coronary flow. (For references, see Wiggers, 18.) Evans and Starling emphasized that considerable variation does occur as did Dusser de Barenne. The seemingly conclusive experiments of Anrep, Blalock and Hammouda (1) when critically examined, show *only* that the flow remained proportional under conditions in which it may be expected that *tension-time* relations of right ventricular pressures were not significantly disturbed. Their experiments clearly showed 1, that the percentile sinus flow remains constant (within 5.5 per cent) when the perfusion pressures are changed, but the beat of the two ventricles remains the same; and 2, that the same constancy (within 8 per cent) obtains when the coronary perfusion pressure is constant, but the left ventricle contracts more vigorously against a higher aortic resistance. In the first instance, no alteration of the relative sinus and Thebesian flow would be expected according to our hypothesis or experiments. In the second series of tests in which coronary pressures were kept constant and arterial resistance was increased, an elevation of left ventricular maximal pressures doubtless occurred, but it is improbable that right ventricular maximal pressures likewise increased; on the contrary there are reasons for believing that they decreased somewhat. As demonstrated by Anrep and Bulatao (2), the pulmonary arterial and hence also the maximal right ventricular pressures in such preparations are determined by the return of venous blood by cardiac veins. Since the coronary sinus flow was led to the exterior, only the

return flow through other communicating veins could have been concerned. The results showed that both the total inflow and the Thebesian flow decreased. Hence initial tension and length in the right chambers would tend to fall and as a consequence also the maximum tension developed during contraction. If this happened, a larger fraction of the reduced total flow would enter the right ventricle and a correspondingly smaller fraction would leave by the coronary sinus. Such predictions actually correspond to results reported in experiments in which contractions of the left ventricle were more vigorous. We believe that our interpretation of such difference in percentile coronary sinus flow is at least as probable as that offered by these investigators, *viz.*, that increased vigor of (left) ventricular contractions compresses sinus veins more than tributaries of the Thebesian system.

The experiments designed to demonstrate that heart rate changes have only a negligible effect on percentile coronary sinus outflow do not seem to be so very crucial. The only observations that we have found rest upon changes in rate after release of heart block due to epinephrine. Since these were either complicated by the actions of the drug or by opposite effects on right ventricular pressure maxima and rate, it is conceivable that other actions interfered. The conclusions that the *same relative coronary sinus outflow* occurs during ventricular standstill or fibrillation as when the heart beats we cannot confirm; nor does it appear to be true in the observations reported. The authors qualify their statement that such constancy occurs only when the ventricles are not dilated. In our experience, however, marked dilatation is a necessary accompaniment of ventricular arrest or fibrillation. But in such cases as much as 50 per cent decrease in relative coronary sinus flow is reported. This they attribute to compression of coronary veins by a weighted heart or to stretching of these veins during dilatation, whereas it is our belief that reduced right ventricular resistance accounts better for the phenomenon.

In 1933, Bergwall and Rühl (5) measured total coronary flow in completely isolated hearts by the difference between the metered outputs of the right and left ventricles, while the coronary sinus flow was measured directly. During cardiac decompensation produced by histamine, the coronary sinus flow changed from 67.6 to 64.5 per cent of the total flow, with a recovery to 68.2 per cent when arrhythmia supervened. Injection of strophanthin led to further decompensation and in addition a reduction of coronary inflow, but the sinus flow remained 64.5 per cent of the total flow. The pressure curves published indicate a great elevation of right and left atrial pressures but under such conditions the right intraventricular pressure maximum could not have become higher despite the ventricular distention; on the contrary they must have been much lower in such hypodynamic beats (*cf.* Fineberg and Wiggers, 11). Such observations are

quite consistent with our conception for any impedance to Thebesian flow during diastole would tend to be compensated by the lower systolic pressure maxima directing less blood to the sinus. Such experiments certainly demonstrate that in special preparations and perhaps also in many conditions of the intact heart, the dynamic circumstances may so counterbalance one another that the coronary sinus flow remains relatively proportional to total coronary flow; but this by no means guarantees such constancy in the normally beating heart or in the heart-lung preparation when right ventricular pressure changes as a result of drugs or nerve stimulations.

We have no direct experimental evidence that stimulation of afferent or efferent nerves or of drugs reputed to affect coronary vessels also affect right ventricular pressures but there is circumstantial evidence that they may do so. Bradford and Dean as early as 1889 showed that a rise in pulmonary arterial pressure could be produced in the dog by stimulation of peripheral ends of cut thoracic nerves (T_2 to T_7) without cardiac acceleration or a systemic arterial pressure rise. Their observations were confirmed by François Franck (1895) and Plumier (1904, 1905). (For references *cf.* Daly, 7). Daly and Euler (1932) demonstrated that stimulation of the stellate ganglion in dogs augments the mean pulmonary pressure as much as 40 per cent while stimulation of the cervical or thoracic vagosympathetic nerves produces a rise in some animals and a fall in others. Such pressure changes in the pulmonary artery could not occur without marked change in the pressure developed within the right ventricle during systole.

The question may be raised, could the elevations of right ventricular pressure resulting from stimulation of vagosympathetic and sympathetic nerves be sufficient to explain the changes in coronary sinus flow found by investigators and attributed to coronary vasomotor changes? Referring back to our experimental results, it is apparent that large increases in right ventricular pressure are not required to augment the coronary sinus outflow considerably. Thus in figures 3B and 4C an increase of only 17 mm. in right ventricular systolic pressure caused a 20 per cent augmentation of coronary sinus flow and a 25 mm. rise occasioned a 40 per cent increase. Since these orders of magnitude correspond to percentile changes often reported as following nerve stimulation and use of drugs, it is apparent that increase in sinus outflow cannot be accepted as evidence that this is due to coronary vasodilatation unless evidence is included that not only arterial pressures but also systolic right ventricular pressure remained constant. Thus stimulation of the stellate ganglion is reported by C. W. Greene (13) to cause an increased coronary flow but according to Daly and v. Euler (8) it produces also pulmonary constriction and an increase in pulmonary mean pressure up to 40 per cent.

Finally epinephrine and strophanthin which augment coronary sinus

outflow also elevate pulmonary arterial and right ventricular pressures although their effects on coronary flow are doubtless the resultant of several actions.

In conclusion, it is not our purpose to deny that interpretations regarding nerve stimulation or drug actions based upon coronary sinus outflow measurements have some factual basis, but we do believe that the evidence presented by others (1, 2) as well as by us requires that such interpretations be submitted to further controls.

SUMMARY AND CONCLUSIONS

By recording the velocity of coronary sinus flow, returned at once to the superior vena cava and calculating the flow per beat and per minute, it was found that: 1, the coronary sinus normally empties into the atrium only during systole; 2, increasing the right ventricular pressure by compression of the pulmonary artery—the heart rate and aortic pressures remaining the same—causes a proportional augmentation of coronary sinus flow; 3, the increase in minute flow following only slight elevation of systolic right ventricular pressure is of the same order of magnitude as that frequently reported from stimulation of cardiac nerves or actions of drugs.

A theory is presented and supported by experiments that the division of coronary return flow between coronary sinus and Thebesian veins is determined not only by the anatomical resistance of these respective paths but by the height to which right ventricular pressure rises during each systole. This accounts for the proportionally larger flow from the coronary sinus in normally beating hearts and the greater drainage by Thebesian vessels in dead hearts. It proves that a greater coronary sinus flow can occur through secondary increase in right ventricular pressure alone.

The conclusion is reached that inferences regarding vasomotor actions in the coronary system based on alterations in coronary sinus outflow cannot be accepted as crucial unless it is demonstrated that right ventricular systolic pressure remained unchanged.

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EFFECT OF CALCIUM AND PARATHORMONE ON SERUM CALCIUM IN NORMAL, ECK FISTULA AND GASTRECTOMIZED DOGS

LUDWIG G. LEDERER AND LATHAN A. CRANDALL, JR.

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago

Received for publication August 7, 1936

A disturbance of calcium metabolism as a result of liver injury has been reported by previous investigators. Walters and Bowler (1) found that $CaCl_2$ injected intravenously produced only about 50 per cent of the normal serum calcium rise in dogs after ligation of the common bile duct. Greenberg (2) and Nitzescu (3) found that intoxication with elementary phosphorus suppressed the effect of parathormone, and more recently Greenberg (4) has found that hydrazine has a similar but less marked effect while carbon tetrachloride is without influence on the serum calcium curve after parathyroid extract. All of these procedures produce relatively acute hepatic injury, and the administration of hydrazine or phosphorus might act by affecting tissues other than the liver. Thomson and Collip (5) have suggested that the action of phosphorus may be secondary to its effect on the bone marrow. We have therefore determined the effect of orally or intravenously administered calcium salts on the serum calcium of normal and Eck fistula dogs. Because Ivy (6) has noted that the vomitus of Eck fistula animals may contain no free acid, a series of gastrectomized dogs was also studied to determine whether the decreased rise in serum calcium that was observed in the Eck fistula animals might be secondary to an anacidity.

METHODS. All animals were kept on a diet of corn meal boiled with bones to which bread was added. The gastrectomized animals (available by the courtesy of Dr. A. C. Ivy) also received ground beef. This diet is adequate to maintain the serum calcium level of normal animals. Serum calcium determinations were made repeatedly on 18 normal and 8 Eck fistula dogs 16 hours after the last meal in order to establish average fasting values. Tribasic calcium phosphate, calcium lactate, and calcium gluconate were given by stomach tube to fasting normal, Eck fistula, and gastrectomized animals, and the serum calcium was followed at hourly intervals for 5 hours. The dogs ranged in weight from 9 to 15 kilograms and were given 10 grams of each calcium salt; since Lieberman (7) states

that this dosage produces nearly maximum absorption from the gastro-intestinal tract in man, we assumed that this amount would bring about a maximum rise in the dog and that little was to be gained by giving a fixed quantity per kilogram. Vomiting or diarrhea were rarely noted; if these occurred that test was discarded.

Calcium chloride was given intravenously to normal and Eck fistula dogs by means of a Woodyatt pump. In one series the dosage was 8.6 mgm. calcium per kilogram, in a second series 17.3 mgm. In both instances the calcium chloride solution was made isotonic by the addition of sodium chloride, and was given at a constant rate for a period of one hour. Calcium determinations were made prior to the injection, half hourly for the first two hours after beginning the injection, and again at the end of the third hour.

Parathormone was injected subcutaneously in fasting normal and Eck fistula dogs in doses of 7 units per kilogram. The serum calcium was determined prior to the injection and every 4 hours thereafter for 24 hours. All calcium determinations were made by the Clark-Collip modification of the Tisdall method (8).

Results. The average fasting serum calcium of the Eck fistula dog is significantly below that of the normal animal (table 1). Oral administration of calcium salts (table 2) was followed in most instances by increases in the serum calcium, but the effect of the same salt varied appreciably in different animals. Tribasic calcium phosphate produced insignificant changes; after calcium gluconate the average maximum increase was approximately equal in the normal and gastrectomized animals, but distinctly less in the Eck fistula dogs. The average serum calcium increase after calcium lactate was almost as great in the Eck fistula dogs as in the normal, while in the gastrectomized animals this salt produced a much greater increase.

The intravenous administration of calcium chloride produced a slightly greater maximum rise in the normal dogs at a dosage level of 17.3 mgm. per kilo, and a distinctly greater rise in normal than Eck fistula dogs when 8.6 mgm. per kilo were given, as is shown in table 3. The effect of parathormone is distinctly greater in normal than in Eck fistula animals (table 4).

Discussion. The general nature of the results indicates clearly that the calcium metabolism of the Eck fistula dog is not normal. This is shown by: 1, a low fasting serum calcium; 2, a decreased rise in serum calcium after calcium salts by mouth; 3, a more rapid removal of calcium from the blood stream after the intravenous injection of calcium chloride, and 4, a decreased effect of parathormone. A consideration of these findings strongly suggests that the Eck fistula dog suffers from a calcium deficiency on a diet which maintains a normal calcium balance in the unoperated animal. Greenberg (2, 4) has proposed the hypothesis that the

liver may be involved as an intermediary in the action of parathormone. It is also possible that a deficiency in the secretion of bile in these animals may lead to inadequate absorption of calcium, which is lost in the stool because of the insolubility of calcium soaps (9, 10). However, since Greenberg found (4) that the effect of parathormone was diminished by only a 2 to 4 day preliminary intoxication with yellow phosphorus in oil, it appears that acute liver injury over a period presumably too short to permit of a significant decrease in the calcium stores will alter the calcium

TABLE 1

Fasting serum calcium in Eck fistula and normal dogs

	NUMBER OF DOGS	NUMBER OF DETERMINATIONS	AVERAGE SERUM Ca	PER CENT BELOW 9.1 MGM. PER 100 CC.
Normal	18	54	10.6 ±0.0646	7.4
Eck fistula	8	34	9.3 ±0.0337	37.0

TABLE 2

Effect of oral calcium salts on serum calcium in normal, Eck fistula, and gastrectomized dogs

	NUMBER OF DOGS	PEAK RISE OF SERUM CALCIUM			TIME OF PEAK RISE
		Average	Maximum	Minimum	
		mgm.	*mgm.*	*mgm.*	*hours*
Calcium phosphate					
Normal	16	0.7	2.5	0.0	1–3
Eck fistula	4	0.5	1.0	0.0	1–3
Gastrectomized	6	0.3	1.0	0.0	2
Calcium gluconate					
Normal	9	2.2	4.8	0.0	2
Eck fistula	6	0.6	1.5	0.0	2–3
Gastrectomized	6	2.1	4.7	1.0	1
Calcium lactate					
Normal	9	1.4	3.8	0.0	2
Eck fistula	6	1.1	2.6	0.2	2–3
Gastrectomized	6	4.3	7.8	2.0	1

metabolism. This favors the view that the effect of liver damage is to change some endogenous factor concerned in calcium metabolism, and not to limit absorption. Calcium excretion has not been studied in these animals; a determination of the calcium loss after hepatic injury would clarify the situation.

It is evident from the studies on the gastrectomized dogs that the absence of gastric secretion does not seriously interfere with the absorption of large doses of calcium. Since the calcium salts enter the small intestine in these animals abruptly and undiluted by the gastric contents, it might be

expected that absorption would occur more rapidly unless other factors act in an opposite manner. A greater rise in serum calcium indicative of more rapid absorption was in fact found after calcium lactate in the gastrectomized dogs. However, the same factors are operative in the case of calcium gluconate, which caused a greater increase in the serum calcium of the normal animal than did the lactate. The greater increase in serum calcium following administration of the lactate in gastrectomized dogs cannot be ascribed to the fact that lactic is a stronger acid than gluconic, since the dissociation constants of the two are almost identical. We cannot offer a satisfactory explanation for the difference between the lactate and gluconate in the gastrectomized animal. The fact that the serum calcium

TABLE 3

Serum calcium after 1 hour continuous administration of calcium chloride intravenously

	NUMBER OF DOGS	AVERAGE MAXIMUM RISE IN SERUM Ca	PER CENT RETURNING TO NORMAL IN 3 HOURS
Given 17.3 mgm. Ca intravenously			
Normal	4	5.0	50
Eck fistula	4	4.4	0
Given 8.6 mgm. Ca intravenously			
Normal	9	2.3	22
Eck fistula	4	1.6	100

TABLE 4

Serum calcium of normal and Eck fistula dogs after 7 units parathormone per kilogram

	NUMBER OF DOGS	AVERAGE MAXIMUM RISE OF SERA Ca	TIME OF MAXIMUM RISE
			hours
Normal	5	1.4	12
Eck fistula	7	0.96	8

rise is higher than the normal in the gastrectomized animal after calcium lactate and equal to the normal after gluconate cannot be taken to indicate that total calcium absorption is as great as in the normal animal, since as might be anticipated the peak of the curve appears earlier. This suggests more prompt but not necessarily more complete absorption. It is evident that it should be possible to produce adequate calcium absorption in the absence of the gastric secretion by the administration of calcium salts.

SUMMARY

Eck fistula dogs exhibit the following abnormalities: 1, a low fasting serum calcium; 2, a lessened rise in serum calcium following calcium lactate

or gluconate by mouth; 3, an abnormally rapid removal from the blood of calcium given intravenously as the chloride; 4, a decreased effectiveness of parathormone in mobilizing calcium. It is concluded that these animals suffer from a calcium deficiency, which may possibly be secondary to a decrease in the amount of bile secreted, or to some endogenous relation of the liver to calcium metabolism.

The rise in serum calcium after oral administration of calcium gluconate to gastrectomized dogs is not significantly less than in normal animals, while that after calcium lactate is greater.

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RESISTANCE OF THE RAT TO HISTAMINE SHOCK AFTER DESTRUCTION OF THE ADRENAL MEDULLA

DWIGHT J. INGLE

From the Section on Biochemistry, The Mayo Foundation, Rochester, Minnesota

Received for publication August 10, 1936

The normal rat is highly resistant to histamine, but the resistance of the adrenalectomized rat to histamine is low. A number of investigators have attempted to evaluate the relative importance of the cortex and the medulla of the adrenal gland in maintaining a normal resistance to histamine. The work of Gottesman and Perla (2) seems to have proved that the administration of cortin to the adrenalectomized rat raises its resistance to histamine. Perla and Gottesman (6) discounted the importance of the medulla, but Wyman (7) and others have found evidence that it plays a significant rôle. The present experiments are part of a series of studies concerning the relative importance of the cortex and medulla of the adrenal glands in maintaining resistance of the organism to various types of stress.

METHOD. Adult female rats were closely matched in groups of from two to four in number on the basis of age and body weight. One animal in each group was kept as a control. Some of the animals were completely adrenalectomized, and the remaining ones were subjected to experimental destruction of the adrenal medulla. Two methods of destruction were used: In some cases destruction was obtained by the spontaneous, complete degeneration of the medulla which occurs when the intact gland is transplanted autogenously to the ovary. The adrenal gland in such cases was fastened to the ovary by a fine silk suture which passed through the body of the gland and around the body of the ovary. The cortex of the gland survived as a permanent graft in 100 per cent of our autogenous transplants whereas the medulla always degenerated. The stage of estrus is not important if hemorrhage of the ovary is avoided, as is easily accomplished by this method.

The second method employed was direct medullectomy by the procedure described by Evans (1). The top third of the adrenal gland was clipped away and the remaining body of the gland was slipped out of its capsule without additional tearing of the capsule or crushing of the gland. The remaining capsule contains only a thin layer of cortical cells that eventually regenerate a mass of cortical tissue approximately equal to the

mass of cortical tissue in the normal gland. All operations were performed as single-stage aseptic exposures of the glands by the thoracolateral approach. Direct loss of blood was consistently avoided. Immediately after operation the animals were placed in a cabinet where the temperature was held constant at 28°C. For seven days subsequent to operation each rat received amounts of cortin adequate to maintain the weight and growth of the completely adrenalectomized animals. At the end of seven days the rats were returned to breeding cages.

The diet used was a commercial preparation containing 1.0 per cent sodium chloride and 0.9 per cent potassium. This diet is adequate for the health, growth and reproduction of normal rats but does not favor the survival of adrenalectomized rats. Only rats from our stock having viable cortical tissue in addition to the infrequently occurring accessory bodies, or those receiving potent extracts of the adrenal cortex, survived for longer than forty days after operation in this laboratory.

After a minimal delay period of forty days the animals were tested for resistance to histamine acid phosphate. A minimum of three days was allowed between tests. The control and experimental animals of any group were always tested together, the time and dosage being constant.

EXPERIMENTS AND RESULTS. A summary of the results of experiments 1, 2, 3, and 5 is given in figure 1. All animals whose adrenal medulla was destroyed showed normal gains in weight after operation and remained free from symptoms of adrenal cortical deficiency at all times.

In experiment 1, the adrenal glands of twelve rats were transplanted to the ovaries; for each of these animals there was a closely matched normal control animal. Intraperitoneal injections of histamine were begun forty days after operation. The initial dosage was 20 mgm. of histamine acid phosphate, and this was increased by 20 mgm. on each consecutive third day until the lethal dose was reached. Ten of the twelve rats in this group succumbed to doses which ranged from 25 to 42 mgm. of histamine acid phosphate per 100 grams of body weight. The remaining two survived doses of 60 mgm. per 100 grams of body weight. No attempt was made to increase the amount of histamine beyond these amounts. All of the normal controls survived and exhibited only mild symptoms of shock.

In experiment 2, ten groups of four animals each were prepared. Each group consisted of one normal animal, one completely adrenalectomized animal, and two animals whose adrenal glands had been transplanted to their ovaries. The completely adrenalectomized animals were maintained in good health with cortin for seven days after operation. Each of these animals then manifested adrenal insufficiency within five to twenty days after the withdrawal of cortin. Before the state of insufficiency had become acute, each of these animals received intraperitoneal injections of

5 mgm. of histamine, and all of them succumbed within an hour after injection. Forty days after operation each of the remaining three animals in each group was subjected to intraperitoneal injections of histamine. One of the transplant animals from each group received an injection of cortin two hours prior to each injection of histamine, the amount being twice the dose of cortin which gives an optimal effect for maintenance of the work capacity of adrenalectomized rats (3). The initial dose of histamine was 20 mgm. and this was increased on every third day by 20 mgm. until the lethal dose was reached for all the transplant group. The range

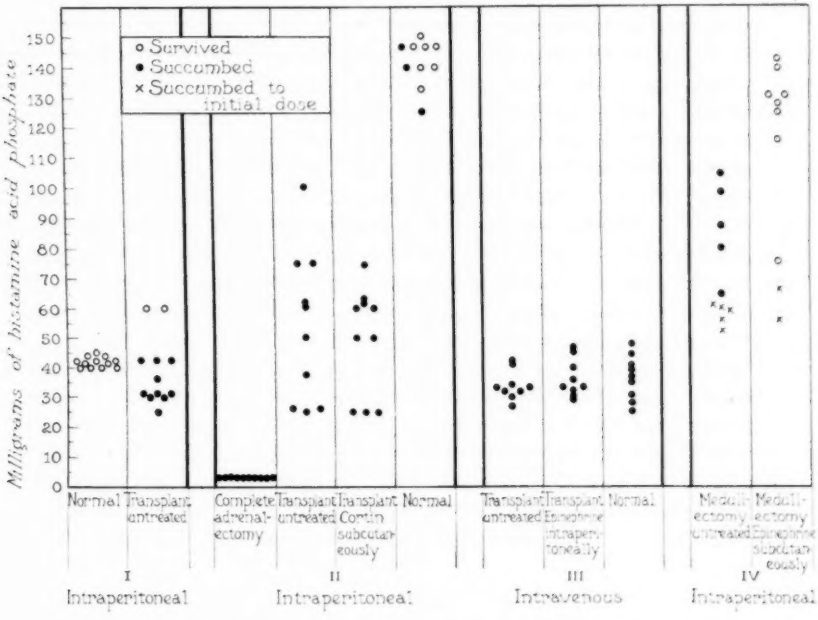

Fig. 1. Maximal amounts of histamine acid phosphate given per 100 grams of body weight.

of lethal dosage for the animals treated with cortin was from 25 to 74 mgm. per 100 grams of body weight, whereas the range for the untreated animals was from 25 to 100 mgm. The minimal lethal dose for the normal group was 125 mgm. per 100 grams of body weight; several of these animals survived larger doses, but the experiment was not continued further.

In experiment 3, nine groups of rats were prepared, each group containing one normal animal and two transplant animals. Injections of histamine were begun forty days after operation. One transplant animal

in each group received 1 cc. of a 1:100,000 solution of epinephrine chloride by intraperitoneal injection five minutes prior to each injection of histamine. The histamine was administered intravenously, being injected slowly into one of the leg veins. The initial dose was 40 mgm. and this was increased by 10 mgm. at intervals of from three to seven days until the lethal dose had been reached for all animals. The range of lethal dosage was from 29 to 47 mgm. per 100 grams of body weight for the transplant group treated with epinephrine, from 29 to 42 mgm. for the untreated animals in the transplant group, and from 25 to 48 mgm. for the normal controls. In this experiment all deaths were invariably preceded by bronchospasm and respiratory paralysis. These deaths occurred within five minutes after the injection of histamine, which is in contrast to a delay of several hours when the histamine was injected intraperitoneally in other experiments of this study.

In experiment 4, six pairs of transplant animals were prepared. After a forty day delay, histamine was injected intraperitoneally on each consecutive third day. One transplant animal in each group also received 1 cc. of a 1:100,000 solution of epinephrine chloride intraperitoneally five minutes prior to the injection of histamine. The injection of epinephrine was repeated every two hours until death or recovery of the animal. An initial dose of 40 mgm. of histamine was given each animal, and this was increased at each test by 20 mgm. The range of lethal dosage for the untreated transplant animals was from 25 to 75 mgm. per 100 grams of body weight. All the animals treated with epinephrine survived the maximal lethal dose for the untreated group. A valid objection to this experiment was pointed out to us by Doctors Bollman and Essex. Since the epinephrine was injected at the same site as the histamine it is possible that the local vasoconstrictor effect of the epinephrine delayed absorption of the histamine. The data for experiment 4 are therefore not represented in figure 1.

In experiment 5, ten groups of rats were prepared, each group consisting of one normal animal and two demedullated animals. After a delay period of forty days, each of the demedullated animals was subjected to intraperitoneal injections of histamine. Normal animals were not tested in this experiment. One of the demedullated animals in each group received 0.25 cc. of 1:5000 solution of epinephrine subcutaneously five minutes before the injection of histamine, and a similar injection at each subsequent two-hour period until death or recovery. Six of the untreated animals and two of the epinephrine-treated animals succumbed to the initial dose of 120 mgm. of histamine. The dose of histamine was increased by 20 mgm. on each consecutive third day until all of the untreated animals had succumbed. The lethal dosage for the untreated animals extended up to 104 mgm. per 100 grams of body weight. Six of

the animals treated with epinephrine survived larger dosages; the maximal lethal dose for these animals was not found.

Necropsy was performed on all the experimental animals. All the transplanted glands were well established and, in the demedullated animals, the cortical tissue had regenerated to a mass roughly equal to that of the normal glands. Sections of a representative number of glands which were stained with potassium dichromate confirmed the extensive observations carried out in this laboratory and others, that both in the transplanted gland and in the demedullated gland, the cortex remained in good repair and the medulla was completely degenerated.

COMMENT. The observations reported here confirm the findings of others that absence of the adrenal cortex lowers the resistance of the animal to histamine shock. Wyman (7) has used the transplant method in studying this problem, but he was unable to demonstrate a difference between the completely adrenalectomized animals and those possessing transplanted cortical tissue. This discrepancy between his findings and the results of the present study has a simple explanation. Wyman fragmented the gland and transplanted it intramuscularly. Unpublished studies on methods for autogenous transplantation of the adrenal glands have clearly shown that, although subcutaneous or intramuscular transplants may maintain life and growth in the rat, they are incapable of providing a sufficient amount of hormone to maintain normal resistance of the animal to stress (work). On the other hand the animal with an intact adrenal gland autogenously transplanted to the ovary exhibits a normal capacity for work (5), a normal level of voluntary activity (4) and a normal resistance to cold and heat (unpublished data). The evidence for a normal quantitative function of these transplants is strengthened further by the demonstration that the animals retain a normal capacity for resisting these forms of stress after unilateral removal of a transplanted gland but quickly lose it on bilateral removal of the transplanted glands.

Although the effect of the presence of viable cortical tissue on the resistance of the rats to histamine shock is clearly established, these animals were inferior to normal animals except when the histamine was injected intravenously. A deficiency due to a lack of medullary tissue is therefore indicated.

Other possible explanations must also be considered. It is conceivable that a condition of adrenal insufficiency which occurred before the establishing of the transplanted tissue or before the regeneration of cortical tissue from the capsule of the gland would produce an irreversible damage to the organism which would permanently lower resistance to histamine shock. Since all the experimental animals were treated with cortin during the first week after operation, and since all were kept free from symptoms

of adrenal insufficiency, it does not seem probable that a severe state of insufficiency was present at any time. There is a possibility that the adrenal cortical tissue in both the transplant and demedullated group remained hypofunctional. However, as previously indicated, animals prepared by these methods retain their ability to work in a normal manner and to resist other forms of stress. The administration of large amounts of cortin to these animals failed to raise their resistance to histamine. Finally, the administration of epinephrine had a strikingly enhancing effect on the resistance of these animals to histamine.

Perla and Gottesman (6) failed to find a beneficial effect of epinephrine on the resistance of rats to histamine shock when the epinephrine was given immediately prior to the histamine. Although the doses of epinephrine used by them and the route of administration were comparable to those in our experiments, their test objects were rats which were manifesting adrenal insufficiency, whereas our own were rats which we presumed to have an adequate amount of adrenal cortical tissue. In the experiments carried out by Gottesman and Perla (2) it was demonstrated that the adrenalectomized rat treated with cortin has a much higher resistance to histamine shock than does the untreated animal. However, they did not maintain a normal resistance in these animals and it may be significant to note that the lethal dosages of histamine in their rats treated with cortin was comparable to the lethal dosages found by us for the transplant and demedullated animals.

The importance of the adrenal cortex for maintaining a normal resistance to histamine shock is confirmed. The evidence that the adrenal medulla also plays a physiologic rôle in protecting the animal against histamine shock is strengthened by the observations reported here.

SUMMARY

Completely adrenalectomized rats showed a low resistance to histamine shock. Animals with the adrenal medulla experimentally destroyed by either demedullation or the autogenous transplant method, but with viable adrenal cortical tissue, showed a resistance to intraperitoneal injections of histamine much greater than that of completely adrenalectomized animals although still inferior to that of normal animals. Intravenous injections of histamine produced death through respiratory paralysis instead of shock and did not permit reliable differentiation between untreated and epinephrine-treated transplant animals and normal animals. The administration of epinephrine increased the resistance of those animals that lacked the adrenal medulla to intraperitoneal injections of histamine, but no additional protection was afforded these animals by cortin.

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A STUDY OF THE SECRETORY NERVES OF, AND THE ACTION OF CERTAIN DRUGS ON, THE PROSTATE GLAND

JAMES I. FARRELL AND YALE LYMAN

From the Departments of Physiology and Urology, Northwestern University Medical School, Chicago

Received for publication August 10, 1936

The physiology of prostatic secretion has been studied only to a slight extent. However, many reports have been made on the pharmacology of prostatic extracts and suspensions of the gland tissue. Such reports are not related to the following study.

Buxmann (1) was perhaps the first to attempt to study the mechanism of prostatic secretion. He found that faradic stimulation with a bipolar electrode applied directly to the gland capsule caused an increase in prostatic outflow which lasted for the period of stimulation. This has been observed by others (2, 3). Farrell (4) using dogs with a "chronic" fistula of the urethra, so prepared that uncontaminated prostatic secretion could be collected, found that the secretion could be augmented by injection of pilocarpine, and inhibited by atropine. This stimulation of prostatic secretion by pilocarpine has been corroborated by others (3, 5). Winkler (3) and others (8, 9) reported that stimulation of the hypogastric nerves temporarily augments the flow of secretion and that stimulation of the pelvic nerve (parasympathetic) causes an intermittent, jet-like discharge of secretion. Waddell (6) and Macht (7) studied the reaction of isolated strips of the gland to drugs. Their results showed that the smooth muscle of the gland in various animals was apparently sympathomimetically innervated, with the exception of the rabbit in which epinephrine and pilocarpine both caused contraction.

In this paper we shall report our observations on the secretory pressure and nerves of the prostate and on the effect of certain drugs on the prostatic secretory mechanism.

METHODS. The dog is well suited for a study of prostatic secretion because he has a large, well developed prostate and no seminal vesicles. Only large dogs about four years old were chosen because it was found that their prostate formed a larger volume of secretion. Nembutal anesthesia was used. All of our dogs were prepared by a method described by Farrell (4) in a previous communication except that a glass cannula was inserted into the urethra after separation of the bladder, for the collection of the

secretion. The vas deferens was ligated to prevent testicular secretion from entering the urethra. Thirty dogs were used in these experiments.

RESULTS. *Continuous secretion.* In all of our experiments we observed a continuous secretion. It was small in quantity, amounting to from 0.2 to 1.0 cc. per hour. The average was about 0.3 cc. per hour. Spontaneous variations were not observed. In the intact animal this continuous secretion, if formed, must regurgitate into the bladder. That such may occur was shown by opening the bladder, evacuating its contents, tying off the urethra distal to the prostate, and then stimulating the hypogastric nerve. Under these conditions prostatic fluid flows into the bladder.

Secretory pressure of the prostate gland and force of contraction of the prostatic musculature. The secretory pressure of the prostate gland was measured by connecting a long glass tube to the cannula. The secretory pressure of four large (40–70 lbs.) dogs was measured, the gland being excited to secrete by the intravenous injection of pilocarpine. The pressure obtained in the different dogs varied from 70 to 120 cm. of prostatic fluid (1.010, sp. gr.) pressure. We believe this pressure is about the true secretory pressure and is not due to contraction of the prostatic musculature, because after the secretion had become stationary at a certain height the injection of a second dose of pilocarpine would cause the pressure to rise for a minute or two after which it would fall to the original level. Further, stimulation of the nerve erigens after the secretory pressure had been reached caused the pressure to rise, for example, from 70 to 101 cm., from 110 to 130 cm., and then to fall to the original level after cessation of stimulation.

To obtain more accurate figures on prostatic secretory pressure and the force exerted by the contraction of the prostatic musculature we used the "capsule method" with rigid tubing, the cannula being inserted into a very small segment of the urethra. By this method the secretory pressure of large dogs varied from 105 to 127 mm. Hg when secreting to pilocarpine. When the secretory pressure had reached its maximum and then the pelvic nerve was stimulated 15 to 25 minutes later, the pressure was augmented, during the period of stimulation, from 17 to 26 mm. Hg. Thus, when the pressure head was 105 to 127 mm. Hg, stimulation of the pelvic nerves caused the muscle of the prostate to support a pressure of from 122 to 143 mm. Hg, which gives an approximate value of the maximum pressure exerted by the prostatic musculature in the dog.

Hypogastric nerves. Stimulation of the hypogastric nerve causes an increase (fig. 1) in the prostatic secretion. This increase continues as long as the stimulation is continued, or for a period of five minutes. During the stimulation a rhythmical wavelike contraction of the prostatic capsule may be observed; but the consistency and color of the gland are visibly unchanged. From 1 to 8 cc. of secretion may be obtained during a five

minute period of stimulation from a large dog. The quantity of secretion is too large to be due to contraction of the gland alone; active secretion by the gland must actually occur. Stimulation of the hypogastric nerve after atropine sulphate (2 mgm.) resulted in no secretion being formed. Stimulation of the nerve after ergotamine, with the resting gland secreting continuously, caused no secretion.

Pelvic nerve (erigens). Stimulation of the nerve erigens caused no increase in secretion; in an occasional animal a few drops of secretion would

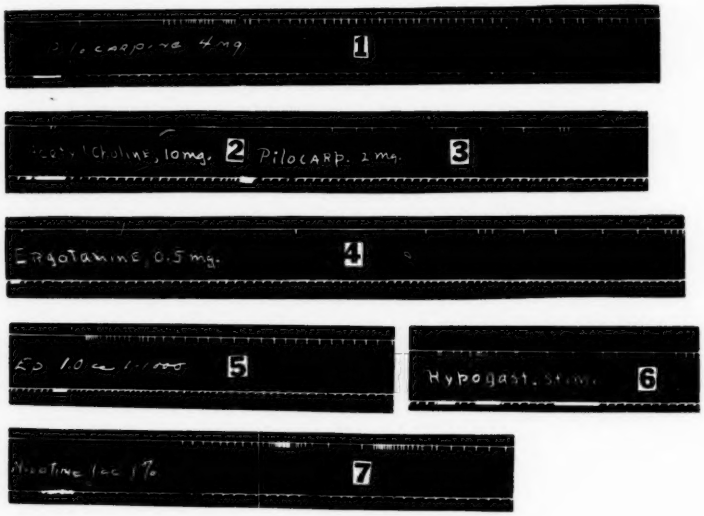

Fig. 1. 1. Four milligrams of pilocarpine hydrochloride injected intravenously. 2. Ten milligrams acetyl-choline injected intravenously. 3. Two milligrams pilocarpine hydrochloride injected intravenously. 4. Five-tenths milligram ergotamine injected intravenously following injection of 2 mgm. of pilocarpine hydrochloride. 5. One cubic centimeter of 1:1000 solution of epinephrine injected intravenously. 6. Stimulation of the hypogastric nerve. 7. One cubic centimeter of 1 per cent solution of nicotine injected intravenously.

be expelled. This is contrary to Winkler's observation, that a jet-like flow results. However, a marked contraction of the prostate gland occurs and its consisteney becomes more firm and any formed secretion contained in the ducts of the gland is expelled. In our experience this never amounted to more than a few drops, which was followed by a subsequent pause in secretion. When the gland is secreting in response to pilocarpine, stimulation of the pelvic nerve causes a temporarily increased output which is followed by a complete cessation of flow for about two minutes. The

period of cessation of flow is too long to be accounted for by filling of the gland on its relaxation. Hence this indicates that the pelvic nerve has a slight inhibitory effect on prostatic secretion. It is too slight and variable, however, to measure quantitatively. The color of the gland did not change appreciably.

Epinephrine. If from 0.5 to 1.0 cc. of a 1:1000 solution of epinephrine is injected slowly intravenously, a prompt increase in the rate of prostatic secretion occurs which continues for about seven minutes. The amount of secretion obtained varies from 1 to 15 cc. The gland contracts and becomes firmer in consistency. Part of the augmentation of the flow may be due to contraction of the prostatic muscle. However, the amount of secretion obtained may exceed the volume of the gland which shows that an increased formation of prostatic secretion results.

Incidentally, it was noted that sperm may pass from the cannulated vas deferens after epinephrine injection.

Histamine. One cubic centimeter of a 1:1000 solution of histamine hydrochloride was injected slowly intravenously and no change in secretion was noted.

Acetyl-choline. Two cubic centimeters of a 1:500 solution of acetyl-choline injected intravenously caused only a slight increase in secretion, although marked salivation was obtained. Thus the gland is quite refractory to acetyl-choline.

Pilocarpine. Two cubic centimeters of a 1:200 pilocarpine hydrochloride solution given intravenously caused a marked increase in secretion after a latent period of about one minute. The increased rate of secretion frequently lasts for several hours. We have collected as much as 150 cc. of prostatic secretion in the course of three hours after pilocarpine stimulation. Stimulation of the hypogastric nerve after pilocarpine tends to augment the flow temporarily, after which a decrease occurs for about thirty seconds; evidently the augmentation is due to contraction of the gland.

Ergotamine tartrate. One cubic centimeter of a 1:2000 solution when injected during pilocarpine stimulation caused a depression of the pilocarpine effect for a few minutes. When the ergotamine was injected with the gland in the resting condition, it had no effect on the secretion of the gland.

Nicotine. The injection of 2 cc. of 1:500 solution of nicotine caused a slight, or, at times, a marked increase in secretion which lasted for about ten minutes. When nicotine is given after pilocarpine, it does not abolish the pilocarpine effect. Nicotine abolishes the effect of hypogastric stimulation for a short period, which confirms Winkler (3).

Atropine. The injection of 1 mgm. of atropine sulfate, slowly intravenously, caused a cessation of the secretion and rendered electrical stimulation of the hypogastric nerve ineffective. It abolished the pilocarpine

effect. Atropine decreases but does not abolish the effect of stimulation of the nerve erigens on the musculature of the prostate, a sluggish response being observed.

Yohimbine. The injection intravenously of 7 mgm. of yohimbine hydrochloride in an aqueous solution caused no change in the secretion of the gland either before or following pilocarpine stimulation.

Discussion. It would appear that the blood vessels of the prostate are not markedly influenced by stimulation of either the pelvic or hypogastric nerves on the basis of our visual observations. This is analogous to the findings of Langley and Anderson (11) for the urinary bladder. To determine this absolutely, some direct method for following blood flow must be employed.

The smooth muscle of the prostate is caused to contract by stimulation of both nerves. Stimulation of the pelvic nerve causes a more marked contraction than stimulation of the hypogastric. This is again analogous to the urinary bladder (11). The hypogastric appears to influence the muscle of the capsule most, and the pelvic nerve all the musculature of the gland, i.e., the stroma and capsule. We observed in no instance any evidence of inhibitory motor nerves. Somewhat like the urinary bladder (11), atropine does not completely abolish the effect of the response of the muscle to stimulation of either nerve, although it markedly reduces the contractile response to stimulation of the pelvic nerve.

Our observations show undoubtedly that the hypogastric nerve contains true secretory fibers for the prostate, as claimed by others (3, 8, 9), and that the increase in outflow resulting from stimulation of that nerve is not due entirely to contraction of the smooth muscle in the gland. Contraction occurs in man (10) as well as the dog. The pelvic nerve contains no secretory nerves. Meager evidence, however, was obtained, indicating that it exerts a slight inhibitory effect on secretion.

Stimulation of the hypogastric nerve during the course of action of pilocarpine augments the flow, but it is difficult to say whether the augmentation is due to secretion or to contraction of smooth muscle.

The effect of drugs on the secretion of the prostate is complicated and difficult to interpret. In general, the prostate responds to drugs much like the submaxillary gland, with the exception that it is somewhat more refractory. Pilocarpine markedly stimulates prostatic secretion, occasionally almost to the extent that it stimulates the salivary glands. Atropine antagonizes pilocarpine stimulation and renders hypogastric stimulation ineffective. The gland is refractory to acetyl-choline but responds to large doses. Epinephrine stimulates the gland to a slight extent. That true stimulation results is indicated by the fact that sometimes the weight of the resulting secretion is greater than that of the gland. Ergotamine

does not stimulate the gland but decreases the secretion caused by pilocarpine which may, however, be due to vasoconstriction. Stimulation of the hypogastric after ergotamine resulted in no secretion. Thus we have a gland whose secretory nerve is apparently blocked by both atropine and ergotamine. Nicotine stimulates secretion slightly and abolishes the effect of hypogastric stimulation. Histamine and yohimbine do not stimulate secretion.

Thus, pharmacologically, the secretory innervation of the prostate is chiefly parasympathetic in nature, although its secretion is also affected to some extent by such sympathomimetic drugs as epinephrine and ergotamine. However, considering the physiologic fact that the pelvic nerve, or the sacral parasympathetics do not augment secretion when stimulated and the sympathetics do, we must consider the prostate as being most analogous to the sweat glands.

CONCLUSIONS

1. The prostate gland secretes continuously, although the amount of secretion is small.

2. The prostate gland, under pilocarpine stimulation, has a secretory pressure of from 105 to 127 mm. Hg.

3. Stimulation of the hypogastric nerve augments prostatic secretion and at the same time causes wavelike contractions of the prostatic capsule.

4. Stimulation of the nerve erigens causes marked contraction of the prostatic musculature which may support a pressure of from 122 to 143 mm. Hg. There are no augmentatory secretory fibers in the nerve, but there is indication of the existence of an inhibitory effect of the nerve upon the secretion under pilocarpine stimulation.

5. Epinephrine, pilocarpine, nicotine and acetyl-choline all caused an increase in the flow of prostatic secretion.

6. Histamine and yohimbine caused no increase in secretion. Ergotamine tartrate has an inhibiting effect on the secretory response to pilocarpine.

7. Atropine antagonizes pilocarpine stimulation and renders hypogastric stimulation ineffective.

8. From the combined pharmacologic and physiologic findings, the prostate may be considered most closely analogous to the sweat glands although it responds to drugs much like the submaxillary gland. It is somwhat more refractory to drugs than the submaxillary gland.

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CARDIAC CHANGES DURING PROGRESSIVE HYPOTHERMIA

JAMES B. HAMILTON, MELVIN DRESBACH AND RUTH S. HAMILTON

From the Department of Physiology, Albany Medical College and Albany Hospital

Received for publication August 14, 1936

In studies of the condition (1) (2) and treatment of hypothermia, the significance of the cardiac changes was apparent. The present investigation is an attempt to evaluate the effect of hypothermic conditions on the heart and the part played by the heart in death resultant from reduced body temperature. These results are not influenced by anesthesia, since a cold environment suffices for temperature reduction in small animals.

METHOD. An ice box kept at 35° to 42°F. was used as the cooling chamber. Animals were fastened upon a small board or restrained in an immobilization cage described elsewhere (3). Body temperatures were obtained by an iron-constantin thermocouple introduced rectally to a depth of 7.5 cm. with suitable precautions for the determination of accurate temperatures (3). At short intervals during the change in body temperature electrocardiograms were taken by lead 2 of 8 rats and 2 kittens with a string galvanometer.

RESULTS. There is an immediate and continued decrease in body temperature following placement of the animal in the ice box at 35° to 42°F. At no time during the decline in body temperature do shivering and thermoregulation prevent the development of hypothermia; instead, the level of body heat is at all times a straight linear decrease directly related to the length of exposure.

Changes produced by temperatures as low as 75°F. (colonic) are not critical. The heart is slowed, the P-R interval lengthened, the amplitude and width of R and T waves increased, and spontaneous movements lost. Temperatures of 65°F. or less are accompanied by rapid and profound disturbances of the nervous, respiratory and vascular systems. Upon release from the cooling chamber the animals spontaneously recover unless the temperature has been lowered to lethal levels of 60° to 54°F. or unless the body level of heat has been maintained for some time at a low level.

A. Rats. Before cooling of the rat, the heart beat varies from 520 to 420 per minute in the different animals, averaging 458 per minute. The slowing of the heart rate follows a direct linear decline until the body temperature is approximately 65°F.; below that the rhythm becomes irreg-

TABLE 1

BODY TEMPERATURE	HEART RATE	RHYTHM	P-R INTERVAL	WIDTH OF R WAVE	WIDTH OF T WAVE	AMPLITUDE OF R WAVE	AMPLITUDE OF T WAVE	P WAVE	S WAVE	Q WAVE	REMARKS
98	460	Reg.	0.04	0.018 0.02	0.06	8	4	Diphasic and monophasic	1 mm.	Presence questionable	
96	420	Reg.	0.04	0.015 0.02	0.06	8 9	4	Majority diphasic	1 mm.	Presence questionable	
93.7	400	Reg.	0.04	0.015 0.02	0.06	10	4 5	Majority diphasic	Absent	Absent	Notching of T becomes more prominent
91.2	380	Reg.	0.04 0.05	0.015 0.02	0.07	10	3 5	Majority diphasic	Absent	Absent	
86	320	Reg.	0.04 0.05	0.02	0.1	10	3 4 5	Mostly monophasic	Absent	Absent	
82	280	Reg.	0.05 0.06	0.02	0.10	9 10	5 6	Monophasic and diphasic	Absent	Absent	
77	220	Reg.	0.06 0.07	0.02 0.025	0.12	10 11	5 6	Monophasic and diphasic	Absent	Absent	T very notched
74	180	Reg.	0.07 0.08	0.025	0.14	13	7 8	Monophasic and diphasic	Absent	Absent	T very notched
71	136	Reg.	0.08 0.09	0.025	0.18	10 11	5 7	Monophasic and diphasic	Absent	Absent	
68	120	Reg.	0.09 0.1	0.02 0.03	0.20	11 12	6 7	Monophasic and diphasic	Absent	Absent	
65	75	Reg.	0.14	0.04	0.2	15	3	At times absent	2 mm. 3 mm.	Absent	Shivering. T becomes much shorter and lower
62	60	Reg.	0.18 0.24	0.03 0.04	0.2	13	2 3	At times absent	2.5 4.0	Absent	
59.5	45	Irreg.	0.22	0.04 0.06 Notched		10 11	2-3	Almost absent	Absent	Absent	Character of beat changes. Ectopic in origin. R notched. Ventricular beat

ular. The slowing of the rate is illustrated by the measurements of rat 8 in table 1. Figure 1 shows the average slowing for the rats with the fall in body temperature, the decrease per minute for every degree fall in the level of body heat being 10.68 beats (between 95°F. and 60°F).

The conduction time increases slowly at first but rapidly as hypothermia becomes more pronounced, especially between 65° and 60°F. (table 1). Figure 2 shows the average and the extremes of the 8 rats. The rhythm remains regular until 65° to 60°F.; thereafter the beat may be ventricular and not regularly correlated with an auricular contraction (table 1). When an irregular rhythm is induced, the conduction is 3 to 6 times that found at normal temperatures.

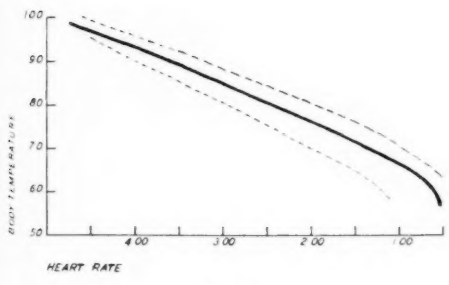

Fig. 1

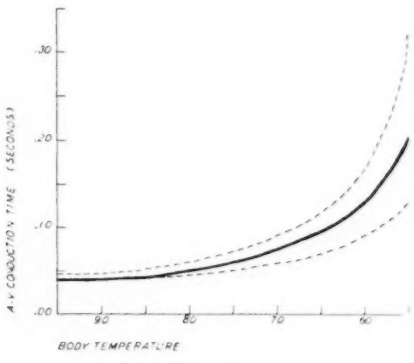

Fig. 2

Fig. 1. The slowing of the heart rate of the rat with the fall in body temperature. ——— average of 8 rats. — — — extremes of 8 rats.

Fig. 2. Lengthening A-V conduction time with progressive hypothermia. —— average of 8 rats; — — — extremes of 8 rats.

In the rise of body temperature subsequent to removal of the rat from the cooling chamber the above changes disappear, the P-R interval shortening, the rhythm becoming regular, the heart rate increasing. At any level of heat the P-R interval is almost identical during cooling and recovery.

The P waves may be monophasic or diphasic or both monophasic and diphasic at the same temperature. The Q and S waves are neither prominent nor constant. The R and T waves broaden with the fall in temperature, R widening from 0.018 second to 0.04 to 0.06 second, T from 0.06 second to 0.20 second (table 1). The amplitude of the R and T waves also becomes greater as the hypothermia progresses (table 1), T tending to become more notched.

At lethal temperatures (60°–54°F.) the ventricular waves are ectopic

in origin and irregular. Beats arise in spurts, accompanied by deflections resembling P waves which occur at about 400 per minute. Within a few minutes the deflections are present only occasionally and are soon entirely absent.

B. Kittens. The same sequence of events characterizes hypothermia in the kitten. The heart rate slows from 300 per minute at normal body heat levels to 60 or less per minute at 65°F. The P-R interval increases from 0.05 to 0.06 second to 0.20–0.30 second, the rhythm remains regular until a level of 65°F. or lower is assumed. The small S wave of 2 to 5 mm. normally present becomes slurred and reduced to 0.5 to 1.0 mm. T and small Q deflections when present are but little changed. The P wave remains either monophasic or diphasic save at low temperatures

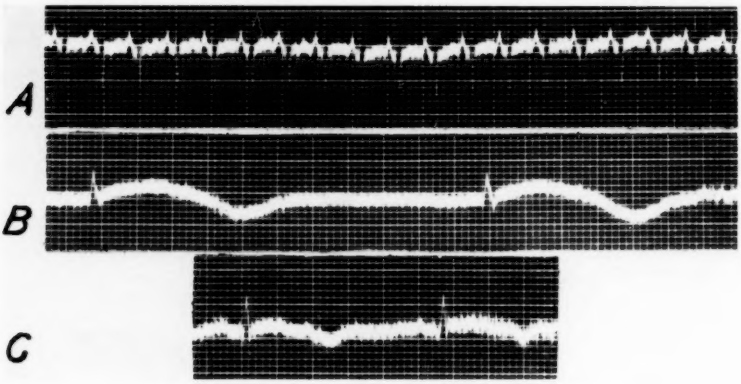

Fig. 3. Electrocardiograms of kitten 1. Previous to exposure to cold: A at 99°F. Severe hypothermia: B at 59°F. During recovery: C at 65°F. A long diphasic ventricular after-phase develops in kittens as hypothermia deepens (B) and disappears as recovery proceeds (C).

when it is inverted. The amplitude of R becomes much greater. A prominent peculiarity as hypothermia develops below 80°F. is a long diphasic ventricular wave, which progressively lengthens, the positive phase increasing, the negative phase decreasing (fig. 3). All these changes are reversible and disappear with elevation of the body level of heat.

Discussion. At no time during the fall in the body level of heat or during maintained low levels is there any indication that the animals reach a state of artificial hibernation as claimed by Simpson (4) and Simpson and Herring (5). Instead, as suggested previously (2) (6), there is ever present in the live animal an attempt to assume normal thermal levels in cold and warm surroundings alike, unless respiratory and cardiac failure intervene.

Cold has a direct effect upon the heart, both in the living animal and *in vitro*. In rats receiving artificial respiration saline was applied directly to the heart through a hole in the anterior thoracic wall. Cold saline reduced the rate to as low as 100 per minute; warm fluid quickened the rate. Alternate cold and warm lavage were used to decrease and increase the rate at will. In the excised heart an inherent thermal sensitivity has been described by Tait. At approximately 60°F. beating ceases in the heart taken from a non-hibernating mammal, whereas in a hibernating animal like the woodchuck temperatures just above freezing may be reached before the heart stops beating (7).

The significance of the vascular changes in the condition of hypothermia cannot be evaluated by electrocardiographic studies alone, but must be supplemented by studies of the volume of blood in circulation and the amount flowing through the various organs, the blood constituents, blood pressure, etc. Yet it is suspected that the cardiac debilities are secondary to anoxemia produced by paralysis of the respiratory centers in the medulla, since at body level of 60°F. 1, the heart beat is 90 or more per minute, strong, and evinces fairly normal electrical characteristics; 2, the descending paralysis of the central nervous system presumably progresses to involve the medullary centers, and 3, respirations occur as infrequently as 1 per minute. Moreover, there is inspissation of the blood, which is to some extent referable to the marked subcutaneous edema.

The electrocardiographic changes incident to non-lethal hypothermia are the converse to those reported in slight hyperthermia (8), (9), (10). Cheer (9) concluded that febrile conditions resulted in acceleration of the heart rate, abbreviation of the systole, and reduction of A-V conduction time.

At lethal elevations of temperature Cheer observed 1, slowing of the S-A rhythm with occasional transfer of the pacemaker to the A-V node; 2, lengthening of the P-R interval; 3, irregularities of rhythm; 4, A-V block and establishment of ventricular rhythm, the auricle contracting only occasionally, and 5, terminal ventricular fibrillation. Save that there was no invariable terminal ventricular fibrillation the final cardiac irregularities in hypothermia agreed with those reported for hyperthermia.

It may be inferred that the S-A node is resistant to both high and low temperatures and to concomitant blood changes; that pacemaker functions reside in the S-A node until the final collapse; that the intraventricular foci are even more resistant than the S-A node; that low temperatures increase conduction time, then result in heart block; that the contraction phase lengthens; that in the course of temperature fall there appear various abnormalities such as change in the amplitude and width of P, R and T waves, inversion of the P waves, slurring, notching and change in the level of the R-T segment, and the development of a lengthy, diphasic

ventricular after-phase in kittens. It is suggested that information regarding the interpretation of changes in the form and duration of the various waves may be gained by further study of the many peculiar changes in the cardiac cycle induced by hypothermia in unanesthetized animals.

SUMMARY

1. In hypothermia produced in rats and kittens without anesthesia there is a linear relationship between the decrease in heart rate and the lowering of the body level of heat.

2. Body temperatures as low as 75°F. are not critical, although there is slowing of the heart to one-third the normal rate, and increase in the conduction time and in the width and amplitude of R and T waves.

3. Temperatures of 65°F. or less are critical; the rhythm becomes irregular, the P-R interval lengthens, and A-V block appears. In kittens there develops a peculiar long diphasic ventricular wave.

4. The cardiac changes are reversible, disappearing as recovery proceeds.

5. Cold has a direct effect upon the heart, but the vascular debilities incident to hypothermia are suggested to be secondary to anoxemia induced by cold narcosis of the medullary respiratory centers.

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STANDARD METABOLISM IN THE WHITE MOUSE[1]

HENRY G. BARBOUR AND JANE TRACE

From the Department of Pharmacology and Toxicology, Yale University School of Medicine

Received for publication August 14, 1936

Although it has frequently been necessary to resort to the use of mice for metabolism work the literature as yet contains no satisfactory statistical study of metabolism in mice reduced to standard conditions. We have had occasion in connection with heavy water studies published elsewhere (1) to devise suitable apparatus for this purpose and to accumulate the results of 126 six-hour runs on 26 normal mice. The low statistical variations obtained may encourage the use of our procedures by others. Further data permit estimation of the influence of a number of variable conditions.

Furthermore, as a result of simultaneous determination of the insensible water loss and weight loss in the six-hour metabolism runs we can evaluate these measurements as criteria for metabolism in mice.

Conventional basal metabolism, it is generally admitted, cannot be determined in the mouse, Benedict and Fox (2) having shown that 10 to 12 minute periods allow the nearest approach to such a determination, which can only be judged from selected experiments. These authors discarded all mice exhibiting muscular activity for more than 15 per cent of the experimental period. In such a selection much hard earned experimental material must be sacrificed and the final results, while agreeing adequately for most purposes, fall admittedly above the conventional basal level.

One is less loath to abandon conventional basal determinations as a criterion for the metabolic rate in consideration of the fact that all so-called "basal" measurements are arbitrary. They do not represent an inviolable "normal" level of cellular activity but rather the degree of chemical exchange going on under some particular condition of nervous tension. Witness for example the "sub-basal" levels found during sleep. The advantage of the conventional criterion lies in the elimination of "voluntary" activity in man and other species capable of training, for admittedly we have no entirely satisfactory quantitative method of allowing for muscular activity.

[1] The expenses of this work were defrayed largely from the Research Fund of the Yale University School of Medicine.

In species not amenable to training one may, however, create a set of conditions under which may be obtained a standard metabolic figure for the normal animal. This must be done by submitting healthy individuals to some invariable daily routine; muscular activity thus becomes adjusted to a constant level. Of course all determinations must be made during the "post-absorptive" condition.

For studies requiring an answer at a given time and in which for other reasons the discarding of data is not feasible we find urgent the establishment of figures for *standard metabolism*. Herewith is presented a study of this sort based upon one hundred and twenty-six six-hour metabolism determinations in mice of standard specifications.

CONDITIONS AND PROCEDURES. We have used only healthy two to four months old individuals of the inbred stock of the Department of Anatomy. The studies have been limited to females, the well known periodic variations in activity due to estrus being eliminated by a preliminary double *ovariectomy*. In this series metabolic determinations were begun anywhere from one to six weeks after operation. The metabolism tests are made in a quiet room free from sunlight and constantly kept at the optimum "neutral" temperature of 28°C. (82°F.).

Our mice received a diet consisting of "Fox Chow" two parts, lard and confectioner's sugar one part each. This was made into a paste to minimize spilling; 1.2 gram per 10 grams of mouse is the standard daily allowance; since some was usually refused the average intake per 10 grams mouse was about one gram of this diet, or one half calorie.

Water was allowed at all times except during the six-hour metabolism period, or more frequently injected in definite dosage (table 1). Mice on the above diet left to determine their own water requirement take about 1.4 cc. per 10 grams. Given, however, by stomach tube, 0.75 cc. per 10 grams per day was found sufficient to keep our mice in good weight and water balance conditions (2). Doses ranging from 0.6 to 2.2 cc. per 10 grams were used (table 1).

Apparatus. Our arrangements not only permit determination of the entire water balance, but also allow separate measurement of the insensible water loss for comparison with the metabolic rate.

The open Haldane metabolism train used has been elsewhere described (3) but some of the technical details and procedures must be here mentioned. The shell caustic used in any convenient vessel at the entrance of the train must be slightly moist; if excess water collects this must be poured off. The H_2SO_4 through which the air is next bubbled should be changed as soon as a few per cent increase in its volume has been noted. Thus the increase of fluid in the safety trap (H_2SO_4-saturated pumice) which follows is avoided. Just before entering the mouse chamber the air passes through a venturi flow meter kept adjusted to a flow rate of 200 cc. per minute.

Experience has shown that the most satisfactory dimensions of the mouse chamber (or funnel) are as follows: height over all, 18 cm.; height of mouse room, 12 cm.; this room is connected with the stopcock below by glass tubing 3.5 cm. in length and one centimeter in diameter over all. From the stopcock up this tube is accurately calibrated in tenths of a cubic centimeter for the measurement of urine. About 0.5 cm. above the outlet of the chamber lies the lower metal platform, made of no. 12 mesh, about three centimeters in diameter, which retains the feces. Mineral oil is introduced above the stopcock to a height of 0.5 cm. above this platform. Thus all excreta are prevented from evaporating. The urine falls to the neck of the chamber and after its quantity is read, can be drawn off by the stopcock free of oil.

The mouse sits upon an upper platform 4.5 cm. above the lower platform which supports it by four metal connecting posts. Approximately this distance is necessary to prevent the tail touching the oil. Since the tail acts as an efficient wick such an accident would ruin the experiment. The mouse platform is of no. 4 mesh. Narrow wire is used in the platforms in order to present less surface for catching and retaining the excreta. Through the stopper at the top of the chamber the inlet tube passes only a few millimeters while the slightly tapered outlet tube extends through the first platform. In this way no part of the body of the mouse can block the outlet. The outlet tube also holds the double platform in place.

It is often desirable to collect all of the insensibly lost water from the mouse. Where this is done the animal is kept on the train practically the entire 24 hours. Except for the actual six-hour metabolism period the mouse is allowed a considerably larger chamber. This consists of a 250 cc. pear-shaped funnel with a large opening through which can be introduced a similar double platform which must be tapered considerably to fit the funnel. Owing to the shape of the funnel and its small opening at the top it may be necessary to build the two platforms closer together than described above, in which case the mouse tail must be protected from the oil by using a finer mesh in the upper platform. The double platform again is held in place by the outlet tube to which also the feeding dish is attached. The collection of "insensible" water from the mouse may of course be done with H_2SO_4 unless it is necessary to recover the water.[2]

The expired CO_2 is collected in an 8 inch U-tube containing a single layer of caustic shells placed end to end. This is followed by a 4 inch U-tube filled with broken shells. On the inlet side of the large tube three drops of water are added just before the first weighing, and one drop

[2] The use of the dry ice method for this purpose is described in detail in a contemporary paper (1).

of water is added to the inlet side of the smaller tube. A second 4 inch U-tube contains H_2SO_4 and pumice for re-drying the air and the three tubes are always weighed together. At the suction end of the train is a constant flow bottle ample enough to contain a column of water at least 10 inches high. Air is allowed to leak in generously through a wide glass tube extending down to the bottom of the bottle. If the inside diameter of this tube be at least one centimeter variations in the suction pressure will not be reflected in the flow meter.

At the beginning of each run it is necessary to test the system for any possible leakage. This is done by suddenly closing the valves at the suction end of the train and examining the effect thereby produced in the first sulphuric acid bottle. Should the bubbling stop suddenly and the fluid rise in the inflow tube, a return toward atmospheric pressure through leakage into the system is indicated. If, however, the bubbling ceases gradually and there is no rise of fluid within the inlet tube it is safe to assume that no leaks are present.

Two separate mouse chambers can be in operation at the same time if the entire train, including the flow meter, is duplicated from the point where the air is first dried. Here a Y-tube connects the H_2SO_4 U-tube to both trains. Both systems may be tested for leakage at one operation.

Our metabolism routine included a seven hour feeding period followed by fasting for at least 10 hours. Thus plenty of time remained for the six-hour metabolism run. Unless water was injected just before the metabolism run and the fasting period it was allowed, by a fountain connected to the feeding chamber under slightly negative pressure, during both the feeding and fasting periods.

Results. The average metabolic figures from twenty-six mice are summarized in table 1 which gives the age of the mice, the month in which the experiments were performed, the methods of water administration and the daily amounts received by injected animals. The average body weight with the mean daily change are also shown. The normal body temperature of most of the mice is included. In the last two columns are given the average calories calculated from the oxygen consumption for each of the twenty-six mice. From three to twelve determinations were made on each animal.

The "standard metabolism" level. Calculated per gram of body weight we arrive from the mouse averages at a mean metabolism of 0.332 calorie per 24 hours. The standard deviation is 0.025, and it will be observed that every animal falls within two standard deviations of the mean. Calculated from 95 individual experiments falling within two standard deviations practically the same result is obtained, namely, an average of 0.330 ± 0.024.

Based on surface area (Benedict's constant 9 was used in the Meeh

formula, $K\sqrt[3]{w^2}$) the mean standard metabolism from the same series of twenty-six mice was 951 ± 30.0 calories per square meter per 24 hours, by mice; by individual experimental runs it was 949 ± 53.3 calories.

The variability between individual mice thus would appear to be 7.6 per cent when calculated by weight, but 3.2 per cent by surface area. While the number of determinations on any mouse was usually too small to determine the variability of the animal from her mean the mice were so standardized that the variability of the 80 or 90 individual experiments must approximate closely what would have been obtained from the same number of experiments upon any one mouse, had it been possible to keep her from exceeding the age limit. This variability was 7.3 per cent by body weight, but only 5.6 per cent by surface area.

Benedict and Fox (2) have summarized a large number of determinations from the literature, which show a surprising degree of discord. Perhaps the only significant work, where periods of several hours are concerned, giving lower figures than ours, on mice exposed to approximately the same environmental temperature, was that of Aszódi (4). His low figures, as has been pointed out by others, are evidently to be ascribed to the fact that his mice were not in a satisfactory nutritional state. This seems clear from the fact that although his thermometer was inserted deeper than ours the temperatures of his mice were usually below 37°C. as against our average normal temperature of 38.1°C. On the other hand our figures are lower than those of many observers because of special very important conditions we have introduced into our procedure. We have, for example, confined the mice to a chamber the size of which is conducive only to very limited movements. Of highest significance is the low degree of variability assured by ovariectomy. The results of Terroine and Trautmann (5) who report 898 calories per square meter per 24 hours in an environment of 33 to 34°C. agree closely with ours.

Age differences, within the limited range of our mice, do not appear to affect the metabolism neither do the variations in the daily water dosage or method of administration (see table 1). Attempts to correlate metabolism with the season of the year (because the walls of the experimental constant temperature room exhibit seasonal temperature differences and could influence metabolism by radiation) were unsuccessful although three January mice gave very low figures. The high metabolism in two of the July mice might have been due to an occasional escape of the room temperature above the 28°C. thermostatic level.

Low food intake and loss of weight. The extent to which metabolism may be influenced by low food intake and loss of weight in mice is indicated in table 2 where are shown results from four mice in which these factors were studied. The metabolism figures under "0 days on low food" are taken from our standard series of experiments given in table 1;

the figures on subsequent days are of course not. They show that mice carried from seven to nine days on from one-half to one-third of their normal food intake show no significant decrease in post-absorptive metabolism. Even five days of complete fasting caused only a slight metabolic

TABLE 1

Standard six-hour metabolism of young ovariectomized white mice

MOUSE	AGE	MONTH	H_2O		BODY WEIGHT		NORMAL BODY TEMPERATURE	METABOLISM		
			Given	Cc./10 grams/24 hours	Average	Mean daily change		Number of determinations	Cal./gram/24 hours	Cal./sq.m./24 hours
	days				*grams*		*°C.*			
F35	103	XII	S	2.0	20.3	−0.08	37.4	5	0.377	(1141)*
B14	97	XI	S	2.0	20.7	−0.25	38.0	6	0.366	(1115)
O1		VII	S	1.1	19.2	−0.2	38.7	6	0.379	(1109)
L76	93	II	F		20.5	−0.25	38.0	4	0.359	(1090)
O2		VII	S	2.2	17.5	−0.25	38.6	5	0.375	(1084)
E30	84	X	S	0.75	19.0	+0.17		4	0.346	(1028)
B42	81	XI	S	2.0	20.2	−0.10	38.6	3	0.334	1009
L71	79	II	F		20.2	−0.1	36.5	4	0.332	1007
E31	84	X	S	0.75	17.6	−0.2	38.8	6	0.339	984
M88	65	IV	O	0.75	15.5	−0.4	36.7	7	0.353	982
O3		VII	S	1.6	16.6	−0.5	38.7	3	0.342	968
M79	59	III	F		18.2	+0.05	38.0	4	0.331	965
N6	125	VI	O	1.2-0.6	20.2	−0.3	38.3	5	0.319	963
N2	120	V	O	1.0	19.2	−0.08		5	0.322	961
D24	88	XI	S	2.0	18.2	−0.2	37.5	3	0.325	953
N1	115	V	O	1.2	19.1	−0.1		6	0.319	948
M80	58	III	F		15.2	−0.1	38.6	4	0.344	947
M84	61	IV	O	0.75	20.1	−0.1	38.2	10	0.315	942
M86	66	IV	O	1.0	16.4	−0.15		3	0.333	941
K67	114	III	F		18.1	−0.05		3	0.320	932
J54	68	XII	S	0.75	18.6	−0.2	39.0	4	0.315	932
K65	79	I	F		22.8	−0.1		3	0.292	926
N4	109	V	O	1.0-0.6	17.1	−0.34	38.4	11	0.324	911
N3	115	V	O	1.2	21.8	0.0		6	0.296	903
K64	75	I	S	1.5	19.4	0.0		3	0.300	902
K63	75	I	S	1.5	20.4	+0.2		3	0.285	(884)
Av.....	77				18.9	−0.14	38.1			
										%
Average metabolism	26 mice.....								0.332±0.025†	7.6
	Individual experiments.....							95	0.330±0.024	7.3
	19 mice.....									951±30.0 3.2
	Individual experiments.....							84		949±53.3 5.6

S = subcutaneous; O = per os (tube); F = fountain.

* Numbers in parentheses excluded from average.

† Standard deviation.

fall in one animal (L67) which, however, was seriously affected by food-lack on the following day.

INSENSIBLE WATER LOSS AND ITS VALUE AS AN INDEX OF STANDARD METABOLISM. The average water loss in standard mice is approximately

300 cc. per square meter per 24 hours. (From eight mouse averages the mean was found to be 304.1 ± 17.1, and from 26 individual experiments, in which the variation did not exceed two standard deviations, the mean was 295.9 ± 18.5 (table 3).) The same data calculated per gram mouse yield an average of 0.1 cc. per 24 hours. As shown also in table 3 the simultaneously determined metabolism averages accord with those previously cited from 19 mice and 84 experiments respectively.

The variability between mice as well as between experiments is shown to be practically identical for the two kinds of measurement. Calculated with reference to surface area it was 5.6 per cent for mice and 6.3 per cent for individual water loss data, while for metabolism it was 5.9 per cent for

TABLE 2

Metabolism after low food intake and loss of weight

MOUSE	DAYS ON LOW FOOD	FOOD INTAKE AVERAGE FOR PERIOD	BODY WEIGHT	CALORIES	
		grams/24 hours	*grams*	*gram/24 hours*	*sq.m./24 hours*
K63	0*	2.17	20.4	0.285	884
	8	1.38	19.9	0.331	996
L67	0*	2.84	23.2	0.282	902
	4	0.75	21.0	0.299	915
	9	0	16.7	0.277	782
	10	0	15.6	0.194	539
M80	0*	1.71	15.2	0.344	947
	7	0.59	12.0	0.335	851
N6	0*	1.82	20.2	0.319	963
	9	0.57	14.6	0.369	998

* Normal averages.

the mice and 5.3 per cent for the individual experiments. On the body weight basis the agreement is nearly as good. This indicates that, each in its own field, the two methods offer the same degree of accuracy.

Correlation of the one method with the other has yielded the results shown in the final three columns of table 3. Evidently the agreement between the averages for any given mouse is far better than between the data for individual experiments. In the former case correlation coefficients of 0.81 and 0.34 were found, in the latter case they were negligibly small.

Metabolism per surface area may be computed from water loss (average 304 ± 17 cc.) by using the correlation coefficient 0.81 and the standard metabolism figure established from 84 experiments, viz.: 949 ± 53 cal-

ories. The formula $x = 0.81 \frac{53}{17} y$ (x = calorie deviation, y = water deviation) gives mouse averages agreeing within the limits −5.3 to +3.2 per cent with those found by CO_2 determinations.

While this method of computation gives evidence of the close correlation between insensible loss and metabolism it offers no great advantage over the use of the simple ratio 3.2:1 derived both from the mouse averages and the experiment averages obtained by the two methods. Multiplying the water cubic centimeter by the factor 3.2[3] gives a mouse range

TABLE 3

Insensible loss as an index of metabolism in standard mice

H_2O			METABOLISM			CORRELATION	VARIATION EXTREMES (BETWEEN CALCULATED AND FOUND METABOLISM)	
n	Average ± σ	Variation	n	Average ± σ	Variation	r	From r	Calories = 3.2 × H_2O
Per square meter per 24 hours								
	cc.	*per cent*		*calories*	*per cent*			
8 mice (45 expts.)	304.1±17.1	5.6	8 mice (45 expts.)	964.4±56.6	5.9	0.81	−5.3%, +3.2%	−4.1%, +6.3%
31 expts.	295.9±18.5	6.3	26 expts.	940.7±49.9	5.3	0.02		−12.4%, +11.4%
			19 mice	951 ±30.0	3.2			
			84 expts.	949 ±53.3	5.6			
Per gram per 24 hours								
6 mice (34 expts.)	0.1020±0.0034	3.3	6 mice (34 expts.)	0.325 ±0.0082	2.5	0.34	−1.8%, +4.5%	−3.9%, +6.9%
35 expts.	0.0985±0.0078	7.9	30 expts.	0.3232±0.0202	6.3	<0.01		−21.3%, +22.6%
			26 mice	0.332 ±0.025	7.6			
			95 expts.	0.330 ±0.024	7.3			

between −4.1 per cent and +6.3 per cent of the found metabolism values; 25 of the 26 individual experiments fell between −12.4 and +11.4 per cent.

The slight apparent advantage for the mouse averages per gram over

[3] Since the calories given off by the water represent 0.58 times the weight of the water the factor 3.2 means that $\frac{0.58}{3.2} = 18$ per cent of the bodily heat is lost by evaporation of water. This is much lower than the proportion (24 per cent) in man (Du Bois, 6).

the surface area calculation is offset by the small number of mice as well as by the lower correlation coefficient. Calculated by mouse weight, greater variability is shown between individual experiments as well as poorer agreement between water and metabolism.

Since five or six water determinations on a single mouse suffice to predict the metabolic rate within six per cent or better, it is obvious that water loss, under our conditions, may be regarded as a satisfactory index of standard metabolism. Indeed merely the weight loss of the mice may serve as a suitable index. Were one to assume a respiratory quotient of 0.73 the weight loss would be identical with the water loss. The average respiratory quotient in the 45 experiments on eight mice summarized in table 3 was 0.76. Since it can be shown that with this R. Q. the CO_2–O_2 difference amounts to 4 per cent of the water loss and since 3.2 less 4 per cent is approximately 3, the simple factor 3 could be used with tolerable accuracy for converting six-hour *weight loss* to calories in mice kept under our standard conditions.

It must, however, be emphasized that water loss and metabolism are by no means parallel phenomena under all circumstances. For example Du Bois (6), Barbour (7) and others have shown otherwise in the onset of fever. Here metabolism and water loss may even vary in opposite directions, at best the water rise lags greatly behind the metabolism rise. As a part of the heat-regulating mechanism of the body the water output varies in the interest of temperature homeostasis (Barbour and Gilman, 8) as when the water loss is decidedly raised in a hot environment while the CO_2 metabolism is not.

Further variation of the water-loss independently of metabolism may be cited from our present studies. All of the above determinations of water loss were made upon mice receiving either 1.0 or 1.2 cc. water per 10 grams body weight per day. In other animals, however, where the water administration was excessive, e.g., 2 cc. per 10 grams per day, the ratio of calories to water sometimes fell as low as 2.5. This is of some physiological interest, indicating that the kidney, in ridding the body of a surfeit of water, may be appreciably aided by the insensible loss mechanisms.

None of the above facts detract from the significance of the water loss as a metabolic index when conditions like environmental temperature and water intake are as clearly defined as in the present report.

CONCLUSIONS

1. It is possible to determine standard metabolism in mice in six-hour runs with a coefficient of variation lower than 6 per cent. This can be accomplished with young adult ovariectomized animals under the particular conditions described.

2. Somewhat better agreement was found by surface area than by body weight. The average metabolism from 84 individual determinations was 959±53 calories per square meter per 24 hours, while 95 individual determinations gave 0.330±0.024 calorie per gram per 24 hours.

3. In mice limited to one-third of their normal food intake for at least seven days, even though a considerable loss of weight is undergone, the post-absorptive metabolism is not necessarily lowered.

4. The six-hour insensible water loss of our standard mice was found to be 300 cc. per square meter per 24 hours, or 0.1 cc. per gram per 24 hours.

5. Water loss per surface area shows sufficient correlation with metabolism to be used as a reliable index of the latter under standard conditions in normal mice. The calories may be calculated as 3.2 × cc. of water lost; or, when the R.Q. may be estimated at 0.76, the body weight loss × 3.0 will indicate the metabolism.

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MAINTENANCE OF ADRENALECTOMIZED DOGS WITHOUT CORTIN, THROUGH CONTROL OF THE MINERAL CONSTITUENTS OF THE DIET

W. D. ALLERS AND EDWARD C. KENDALL

From the Section on Biochemistry, The Mayo Foundation, Rochester, Minnesota

Received for publication August 15, 1936

During the past thirty-eight years a large amount of work has been carried out on adrenalectomized animals in regard to the effect of sodium chloride. Beneficial effects have been noted by all investigators but the results have all been consistent in regard to the impossibility of maintaining adrenalectomized dogs in a normal condition through the addition of sodium chloride to the diet. The literature on this subject was reviewed by Loeb in 1935.

About eighteen months ago work was undertaken in this laboratory to investigate further the influence of mineral metabolism on the survival time of adrenalectomized dogs. Because of the suggestion of Hastings and Compere that potassium might exert an unfavorable influence, the diet chosen was low in potassium. Sodium chloride in amounts varying from 8 to 20 grams a day was added to the diet of two dogs which had been adrenalectomized. In agreement with previous observations it was found that after from thirty to sixty days the dogs lost their appetites. This was followed by persistent vomiting, a decrease in weight and obvious symptoms of adrenal deficiency. At this time it was found that the value for the bicarbonate of the blood was only 30 volumes per cent. This indicated that a mild acidosis had developed in the animals and suggested the addition of sodium bicarbonate to the diet. When this was done the dogs' appetites returned and the animals were able to retain food. Because of the possible irritating effects of sodium bicarbonate, sodium citrate was substituted. With the combination of sodium chloride and sodium citrate one dog was maintained for eighty-four days and the other for one hundred fifteen days without cortin (figs. 1 and 2).

From time to time variations were made in the amounts of sodium chloride and sodium citrate which were added to the diet. During an interval in which the sodium chloride was reduced to a low level one of the dogs passed into a crisis of severe adrenal deficiency and died. At the end of one hundred fifteen days the experiment was terminated as the remaining dog was in excellent condition and it did not seem desirable to prolong the experiment.

After it had been shown that dogs could be maintained with a diet high in sodium chloride and sodium citrate, three other dogs were treated in the same manner (tables 1, 2 and 3). However, it was not possible to carry these three dogs through the strain of operation without cortin. They were therefore treated with cortin for several days immediately after operation. The administration of cortin was then stopped. Each of the dogs had the typical symptoms of adrenal deficiency. They were brought back to normal by the administration of sodium chloride and cortin. The

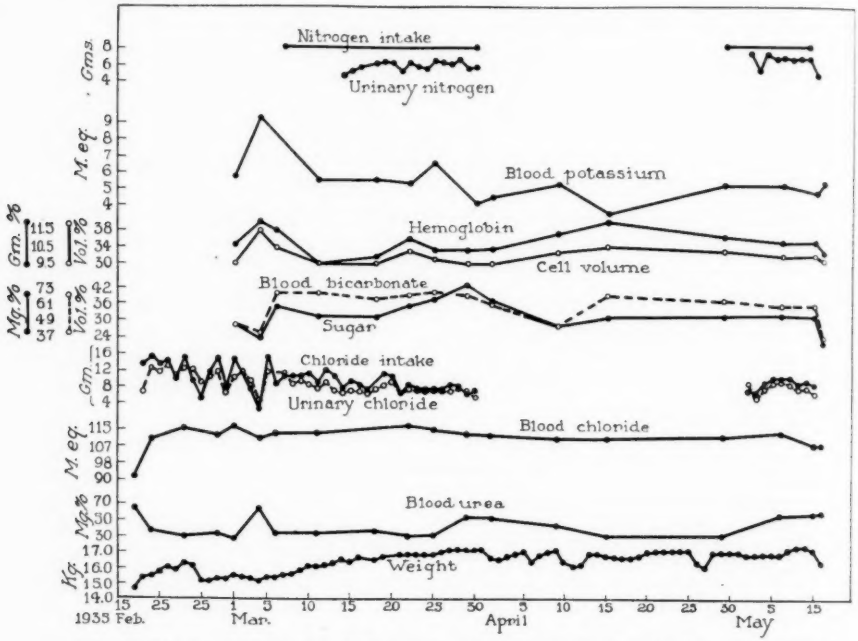

Fig. 1. Biochemical study of bilaterally adrenalectomized male dog maintained eighty-four days without cortin by the administration of sodium chloride and sodium citrate.

cortin was withheld and the dogs were maintained with sodium chloride and sodium citrate only. It was impossible to maintain one of these animals with sodium chloride and sodium citrate longer than two weeks after the administration of cortin was stopped. It was then found that this dog was badly infested with intestinal parasites. These were removed and the dog was maintained with sodium chloride and sodium citrate in an entirely satisfactory condition.

The adrenal glands of all of the dogs were removed under aseptic surgical

technic and ether anesthesia. Examination of the animals after death has shown that the adrenal glands were completely removed in each instance. If these animals were maintained on a diet which did not contain more than 2 grams of sodium chloride a day there was a rapid increase in the value for the blood urea within three days after the administration of cortin was stopped. When the dogs were maintained with sodium chloride and sodium citrate there was an even more rapid rise in the value for the blood urea when the administration of salt was stopped. A typical curve given by one of the animals is shown in figure 3. The level of the blood urea was

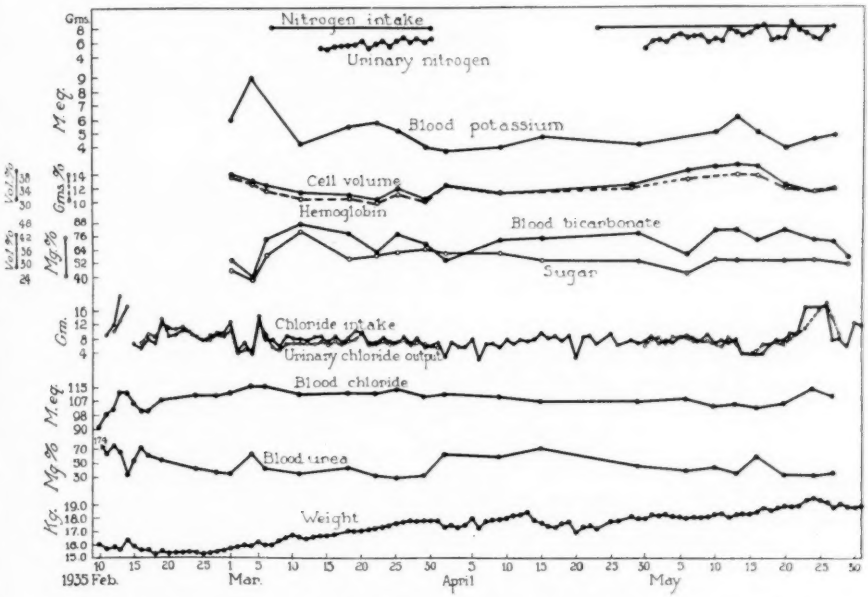

Fig. 2. Maintenance of a bilaterally adrenalectomized female dog for 115 days without cortin by the administration of sodium chloride and sodium citrate.

promptly restored to its former value by the administration of sodium chloride and sodium citrate, without the use of cortin.

It has been found that when there are severe symptoms of adrenal deficiency it may be necessary to give large amounts of cortin with the salt in order to save the animal. It has been our experience that it is possible to restore to a normal condition animals which have a high degree of deficiency, if they are treated with large amounts of sodium chloride, sodium citrate and cortin. The irreversible condition described by Hartman and others applies, if at all, to an extremely small group of animals,

and it seems probable from our results that if the animals are given proper treatment none of them will pass into a condition which can be described as irreversible.

It is necessary to provide a diet which is appetizing. This may require a change from time to time in order to please the appetite of the animal. If, however, the dog refuses to eat we have found it necessary to resort to forced feeding in order to maintain not only the intake of sodium chloride and sodium citrate, which were always given with the food, but to provide the necessary caloric intake and to maintain the blood sugar at a normal level. We have not found the value for the blood sugar below normal in

TABLE 1

Maintenance, by administration of sodium chloride and sodium citrate, of dog 3 which had its second adrenal gland removed June 25, 1935 and received cortin until July 8

DATE	WEIGHT	ADDED TO FOOD		BLOOD UREA	PLASMA CHLORIDE	PLASMA HCO_3	COMMENT
		Sodium chloride	Sodium citrate				
1935	*kgm.*	*grams*	*grams*	*mgm. per 100 cc.*	*m. eq. per l.*	*volume per cent*	
7–8	9.9	6	5	39	104.3	39.0	Cortin stopped. Given 0.5 per cent NaCl in drinking water
7–10	9.8	6	5				Condition excellent
7–17	9.8	6	5				Condition excellent, appetite good
7–24	10.6	6	5	32	102.5	48.6	Condition excellent
8–2	10.5	6	5	48	102.5	41.2	Condition excellent
8–4	11.2	6	5				Condition excellent
8–10	11.0	6	5	48	102.5	48.0	Condition excellent but refused food. Force fed
8–14	11.4	6	5	48	104.3		Refused salt water. Given tap water
8–19	12.4	6	5	46	107.8	40.3	Condition excellent
8–22	12.6	6	5	36	110.2	39.1	Condition excellent

any of these animals. It has been observed, however, that if an intravenous injection of glucose is given this may be followed within an interval of two or three hours by severe hypoglycemia. Provided the dog has food in the alimentary tract, this usually is not severe or of long duration and the animal will survive. If the animal has not eaten within eighteen hours and an intravenous injection of glucose is given, the hypoglycemia which follows may be the apparent cause of death. This was true in two of the animals of this series and in a third case the animal was restored to normal only after vigorous and long continued treatment. The lowest value for the blood sugar determined in this animal was 19 mgm. per cent. The animal was restored by a continuous intravenous injection of sodium

chloride, sodium bicarbonate and glucose for a period of eight hours. At the end of this time the dog was given cortin and, although relieved of the critical condition by this treatment, it was about seven days before the appetite was restored to normal. During this time the treatment consisted of daily injections of cortin and the administration of sodium chloride and sodium citrate by stomach tube and the addition of sodium chloride and sodium citrate to the diet which was forced.

TABLE 2

Maintenance, by administration of sodium chloride and sodium citrate, of dog 4 which had its second adrenal gland removed June 20, 1935 and received cortin until June 28

DATE	WEIGHT	ADDED TO FOOD		BLOOD UREA	PLASMA CHLORIDE	PLASMA HCO_3	COMMENT
		Sodium chloride	Sodium citrate				
1935	*kgm.*	*grams*	*grams*	*mgm. per 100 cc.*	*m. eq. per l.*	*volume per cent*	
6–28	16.3	3	3	30	116.2	42	Stopped cortin. Given 0.5 per cent NaCl in drinking water. Condition excellent
7–5	15.7	3	5				Condition excellent
7–6	14.8	3	5				Condition excellent
7–8	14.7	3	5	45	105.9	37.2	Fairly active. Sodium chloride in food increased to 5 gm. daily
7–11	14.3	0	0				Hot day, refused food, was weak. Went into crisis. Intravenous injection of NaCl, $NaHCO_3$, glucose and cortin
7–14	13.9	5	5				Condition improving, appetite poor. Given cortin
7–17	14.4	5	5				Appetite improved. Given cortin
7–20	15.5	6	5				Cortin stopped. 0.7 per cent NaCl in drinking water
7–24	15.0	6	5	30	102.5	50.4	Condition normal
8–1	15.7	6	5				Weak. Force fed
8–2	14.7	0	0	59	109.5	35.6	Dog in crisis, given cortin
8–20							Condition excellent; cortin continued since 8–2. Given an anthelmintic and cortin
8–23							Given an anthelmintic. Cortin stopped
8–30	17.8	6	5				Condition excellent. 0.8 per cent NaCl in drinking water
9–6	18.0	7	5	44	102.5	39.0	Condition excellent, appetite good, very active
9–23	19.9	7	5	39	119.5	37.5	Condition excellent

Throughout these experiments the diet was varied both in quantity and

quality. It was always adequate in regard to calories and vitamins and consisted in various proportions of casein, sucrose, lard, butter fat, agar agar or bone ash, dried yeast, and, occasionally, commercial canned dog food. It was found that 6.5 mgm. of magnesium per kilogram of body weight and 540 mgm. of calcium per day were adequate. Occasionally, a small amount of lean meat was given in order to please the appetite of

TABLE 3

Maintenance, by administration of sodium chloride and sodium citrate, of dog 5 which had its second adrenal gland removed June 20, 1935 and received cortin until June 28

DATE	WEIGHT	ADDED TO FOOD		BLOOD UREA	PLASMA CHLORIDE	PLASMA HCO_3	COMMENT
		Sodium chloride	Sodium citrate				
1935	*kgm.*	*grams*	*grams*	*mgm. per 100 cc.*	*m. eq. per l.*	*volume per cent*	
6-28	14.9	3	5	25	111.1	40.2	Stopped cortin; condition excellent; 0.5 per cent NaCl in drinking water
7-8	14.3	3	5	45	102.5	37.2	Condition excellent
7-10	14.0	6	5				Condition excellent
7-17	13.9	6	5				Condition excellent
7-24	14.6	6	5	30	102.5	41.2	Condition excellent
8-2	16.5	6	5	26	116.2	39.2	Condition excellent
8-10	17.1	6	5	38	119.5	39.3	Condition excellent
8-14	17.4	6	5	36	109.5	38.4	Condition excellent
8-31	18.4	0	0	40	116.2	35.0	Condition excellent. Sodium salts withdrawn from diet
9-3	17.2	0	0				Restless, vomiting
9-4	16.8	6	5	200	99.1	29.0	Apathetic and weak. Was force fed but vomited entire ration. At 3 p.m. condition was critical; given an intravenous injection of 30 gm. glucose, 7 gm. NaCl and 3 gm. $NaHCO_3$
9-5	17.4						Active. Ate most of diet
9-6	17.9	6	5	46	111.1	36.0	Condition normal; ate all of diet
9-9	18.4	6	5	54	107.8	38.0	Condition excellent

some of the dogs but this was not continued for more than a short interval. Although the maintenance of some of the dogs with sodium chloride and sodium citrate and without cortin was carried out for only a few weeks, we regard the result as significant because of complete loss of appetite, loss of weight, and increase in the value for the blood urea which followed within four days when sodium chloride and sodium citrate were not added to the diet.

While this problem was under investigation, Harrop and co-workers treated adrenalectomized dogs in a similar manner. Their results, however, differed from ours in two important respects. One difference is in regard to the administration of the sodium salts. Harrop and co-workers gave their salts by stomach tube twice a day. This requires a large expenditure of time and is not necessary. We added the sodium citrate and 6 grams of sodium chloride to the daily diet. In addition to this, sodium chloride was added to the drinking water of the dogs to form a 0.6 per cent solution which furnished 4 to 10 grams more of sodium chloride

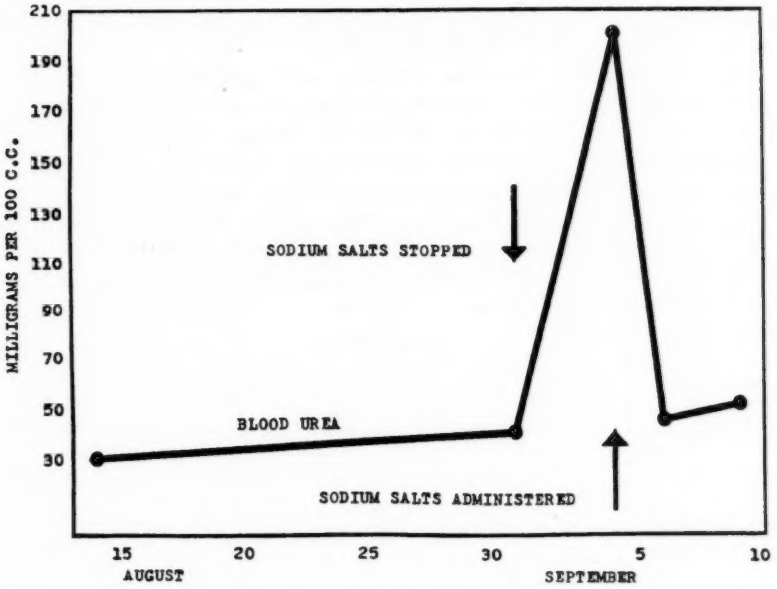

Fig. 3. Effect on blood urea when sodium chloride and sodium citrate are withheld from a bilaterally adrenalectomized dog maintained without cortin.

a day, depending on the amount of water taken. By the administration of sodium chloride and sodium citrate in the food and water the care of these animals is no greater than that required for the ordinary experimental animal.

Harrop and co-workers reported that their dogs gained in weight and were apparently in normal condition. However, the concentration of electrolytes in the blood was not normal and they state that "it is only by the exhibition of both extract and salt in adequate amounts that entirely normal plasma electrolyte levels may be sustained in the totally suprarenalectomized dog."

Harrop and co-workers did not use a diet which was either low or uniform in its content of potassium, and it appears probable that the reason why the concentration of potassium in the blood of their dogs was high was because of the potassium contained in the food. The importance of a rigid control of the intake of potassium will be shown in another paper. With the diet which was used in our experiments the potassium content of the serum could be reduced even below the normal figure for the dog. The minimal requirement of potassium has not been determined but the total potassium in the daily diet of these dogs was less than 200 mgm. The dogs in our series all gained weight; they were active, strong and vigorous. The concentrations of urea, glucose, sodium, potassium, chloride and bicarbonate in the blood were all within normal limits.

PROTOCOLS. The following methods were used: for total nitrogen, Kjeldahl's method; for magnesium, the Fiske and Subbarrow modification of the method of Kramer and Tisdall; for calcium, the Clark and Collip modification of the method of Kramer and Tisdall; for potassium, the chloroplatinate method as modified by Shohl and Bennett; for sodium, the method of Barber and Kolthoff; for chlorine, the method of Wilson and Ball; for carbon dioxide, the method of Van Slyke; for glucose the improved Folin-Wu method (1929); for urea, the method of Van Slyke and Cullen as modified at The Mayo Clinic.

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RENAL BLOOD FLOW OF UNANESTHETIZED RABBITS AND DOGS IN DIURESIS AND ANTIDIURESIS[1, 2]

ARTHUR M. WALKER, CARL F. SCHMIDT, KENDALL A. ELSOM AND CHARLES G. JOHNSTON

From the Departments of Pharmacology and Research Surgery, University of Pennsylvania

Received for publication August 18, 1936

The renal blood flow of unanesthetized rabbits and dogs has been measured in these experiments during the changes in urine volume produced by the ingestion of water, by pituitrin, by a xanthine and a mercurial diuretic. It was hoped that such measurements would aid in determining whether the urinary changes were due to alterations in the amount of glomerular filtrate or in the amount of fluid reabsorbed by the renal tubules. The experiments have been extended to include the measurement of glomerular filtrate by the indirect method of creatinine clearances with the expectation that the two methods of approach might, in combination, allow a precise answer to the question. Neither method has provided definite evidence of alteration in glomerular function and we are therefore of the opinion that the changes in urine volume are chiefly due to changes in tubule activity.

METHODS. *Stromuhr.* The thermostromuhr introduced by Rein in 1928 (1) offered the first practicable way of measuring blood flow in unanesthetized animals, but difficulties in its management have prevented its general use. Because of difficulties with the Rein apparatus the thermostromuhr used in these experiments (2) was developed. It differs somewhat from that of Rein in principle and has the advantage of utilizing the more easily controlled current of a storage battery rather than a high frequency current as the source of heat. A constant current of two amperes, passing through a strip of resistance metal, warms a silver plate lying at the bottom of a trough in a bakelite block to a temperature higher than that of blood. A blood vessel lies in this trough in immediate contact with the silver plate. As the flow within the vessel

[1] Reported before the American Society for Pharmacology and Experimental Therapeutics at Washington, D. C., March 26, 1936 (J. Pharmacol. Exper. Therap. **57**: 146, 1936).

[2] The expenses of this investigation were defrayed in part from a grant by the Commonwealth Fund.

increases the plate becomes cooler, as it decreases the plate becomes warmer. These changes in temperature are recorded by a galvanometer which is connected to two thermal junctions, one formed by the silver plate and a constantan wire (the "hot junction"), the other (the "cold junction") lying proximally to it at the other end of the bakelite block or in a separate block on a nearby blood vessel. The deflections of the galvanometer correspond to the temperature difference between the junctions and consequently provide a record of blood flow which is registered manually on a smoked drum. A more detailed description of the stromuhr is given elsewhere (2).

Installation of stromuhr. a. *Rabbits.* Stromuhrs were installed in 21 male rabbits weighing from 2.1 to 2.6 kgm. Installations were made without aseptic precaution, under paraldehyde or urethane anesthesia in 7 of the earlier experiments, under local anesthesia in the later experiments. The small size of the renal artery necessitated placing the stromuhr on the abdominal aorta, which had therefore to be prepared so that it supplied blood only to the left kidney: with the animal on its right side the aorta was exposed retroperitoneally, without traction on the kidney or pressure on the left renal artery, and was ligated immediately below the left renal artery; between this point and the superior mesenteric artery a space for the stromuhr was cleared by ligating and cutting the right renal artery and such lumbar branches as were present. A stromuhr 8 mm. long with a trough 2 mm. in diameter fitted the aorta well and was firmly immobilized between vessel and paravertebral muscles. The wires which issue from its side were anchored to the muscles, the incision closed, and the experiment begun immediately. The animals remained quiet, without obvious discomfort, during the preparation and subsequent observations.

b. *Dogs.* Stromuhrs were installed in 33 female dogs weighing from 7.9 to 18.0 kilograms after inactivation of the contralateral kidney by renal artery ligation (7 instances) or by nephrectomy (26 instances). In 10 animals installation was on the right renal artery, in 20 animals on the left; in 3 a stromuhr specially designed to imitate the conditions of the rabbit experiments was placed on the abdominal aorta after its preliminary ligation below the origin of the left renal artery. The installations were made with aseptic precautions, under paraldehyde or pentobarbital anesthesia, the renal artery being exposed and cleared down to its junction with the aorta and the stromuhr applied at that point. The course of the renal artery made it difficult to immobilize the stromuhr by anchoring it to paravertebral muscles or aorta and no one of the attempts to effect this by altering the design of the stromuhr was entirely successful. The experiments were performed from 15 to 72 hours after installation.

Procedure. Both rabbits and dogs were constrained to lie on their right sides. Urine was obtained by an inlying bladder catheter. Continuous

records of renal blood flow and urine flow were made during the entire experiment which lasted an average of 4 hours in rabbits and 6 hours in dogs. Control periods of 30 minutes in rabbits and 1 to 2 hours in dogs preceded the administration of water which was given repeatedly by stomach tube in 50 to 100 cc. doses to rabbits and 300 to 500 cc. doses to dogs. Diuresis occurred more uniformly in rabbits when the animals were given large amounts of fluid orally during the 24 hours preceding operation. Creatinine clearances were measured in 11 rabbits and 21 dogs, solutions of creatinine being injected intraperitoneally 30 minutes or more before beginning the first clearance period in dosages of 1 gram to rabbits

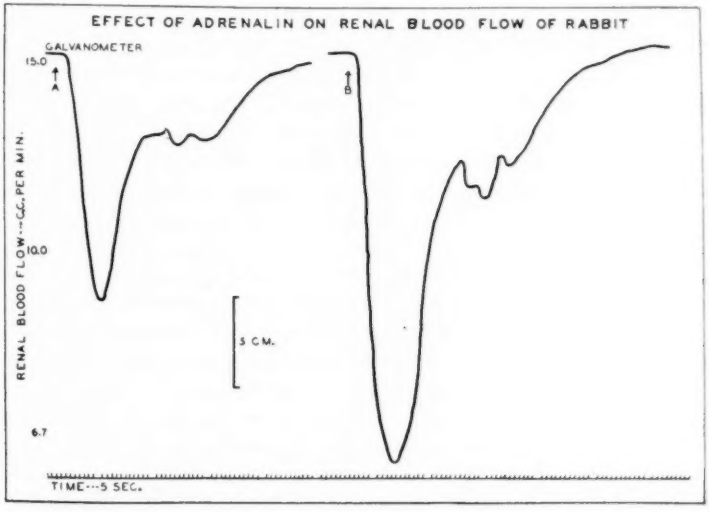

Fig. 1. Tracings showing deflections of galvanometer produced by intravenous injection of (A) 0.07 cc. and (B) 0.15 cc. 1:10,000 adrenalin hydrochloride into an unanesthetized rabbit. Stromuhr subsequently calibrated.

and 3 grams to dogs. In rabbits such injections were followed by a brief diuresis. Blood was taken at the middle of each clearance period in 2 cc. samples from the ear vein of rabbits and in 10 cc. samples from the jugular vein of dogs. The usual precautions were taken to assure emptying the bladder at the beginning and end of each period. When the water diuresis had fully developed, pituitrin (obstetrical, Parke-Davis) was injected subcutaneously. Early in the resulting antidiuresis, or during a period of low urine flow when pituitrin had not been used, theophylline-ethylenediamine (Thephyldine-Kretschmar) was injected intravenously into rabbits and salyrgan (Metz) into dogs. Within an hour after the

development of diuresis the animals were anesthetized with pentobarbital, ether or chloralose and preparations made for calibration.

Adrenalin was injected intravenously before anesthesia was induced in 2 rabbits and 15 dogs to demonstrate the sensitivity of the stromuhr to decreases in renal blood flow. Typical results of these injections appear in figure 1.

Calibration of stromuhr. Since the temperature difference between the two thermal junctions depends not only upon the volume of blood flow but also upon the thickness of the vessel wall, the perfection of contact between vessel and silver plate, and the diameter of the vessel—factors which vary from animal to animal—we found it essential to calibrate the instrument at the end of every experiment.[3] For this purpose the galvanometer reading was compared with the volume of blood flowing from the kidney into a pipette in the vena cava (method of Barcroft and Brodie (3)): the animal was anesthetized and eviscerated, the stromuhr remaining in the retroperitoneal position occupied during the experiment; blood flow was repeatedly raised by the injection of hypertonic sucrose or thiosulphate solutions and lowered by the withdrawal of blood, and the deflection of the galvanometer at numerous levels of blood flow was charted (fig. 2). No experiment was accepted unless such a calibration was performed and the stromuhr was thus proved to be capable of reflecting changes in blood flow. It can be said with assurance that changes in blood flow of the order of 5 per cent have not escaped detection since, in 15 acceptable experiments on rabbits and 21 on dogs, a 5.5 per cent increase in blood flow produced, on the average, a 1 cm. deflection of the galvanometer. Since the chief conclusions from our experiments depend on changes in blood flow rather than on the actual volume of flow, this demonstration of sensitivity provides sufficient basis for their acceptance. In rabbits no factor other than changes in blood flow appeared capable of affecting the galvanometer and each calibration therefore provided absolute figures for blood flow throughout the whole of the preceding experiment. In dogs two sources of error were discovered which affected the accuracy of the figures for actual blood flow: 1. Early in a number of experiments a gradual drift of the galvanometer reading occurred in the direction of increased blood flow. Its extent made it clearly an artefact. It was proven not to be due entirely to direct heating of the upper ("cold") junction since it occurred when the two junctions were completely separated. This drift had the effect of making the blood flow figures obtained at calibration inapplicable to the early part of the experiment unless measurements were delayed until it had ceased. 2. Permanent deflections of the galvanometer occurred in 13 of

[3] The expectation (2) that a calibration on a single vessel in one animal would prove applicable to the same vessel in other animals has proved unfounded in the circumstances of the present experiments. The absolute position of the galvanometer at any given blood flow varied from animal to animal.

the 21 experiments on dogs at the moment of movements by the animal or of manipulations during the preparation for calibration. These deflections were due, it is believed, to changes in the contact between vessel wall and silver plate consequent upon incomplete fixation of the stromuhr. The figures for actual blood flow are not accurate in experiments in which such deflections occurred, and are not given consideration in our discussion.

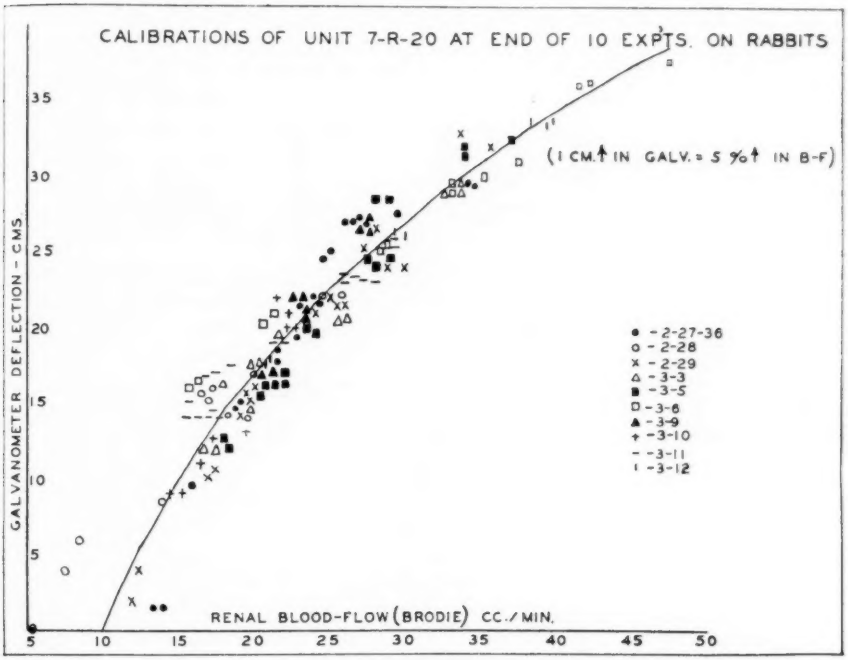

Fig. 2. Results of calibrations performed at the end of 10 experiments on rabbits. The curve is so drawn that a 1 cm. deflection of the galvanometer corresponds to a 5 per cent increase in blood flow. The data of each calibration have been shifted laterally till one point fell on the curve in order to correct for the differences in base-line of the galvanometer existing in individual experiments. The manner in which the data fit the curve indicates the sensitivity of the stromuhr in the preceding experiment.

WATER DIURESIS. *Results.* Renal blood flow was recorded during the changes in urine volume produced in a single kidney of 16 rabbits and 20 dogs by the oral administration of water.[4] Definite diuresis resulted in all

[4] Five experiments with rabbits and 13 with dogs are omitted from consideration because the sensitivity of the stromuhr was either not investigated or was absent at the end of the experiment. There was nothing in their results at variance with the findings to be described.

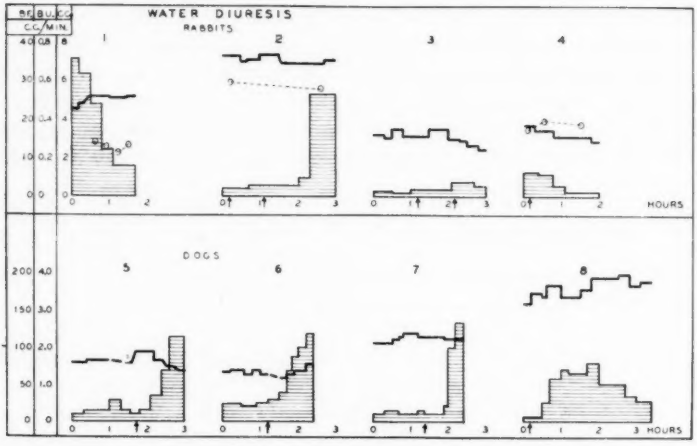

Fig. 3. Changes in renal blood flow (solid line, B.F.), bladder urine (shaded column, B.U.), and creatinine clearance (open circle, C.C.) produced by the administration of water by mouth (arrow).

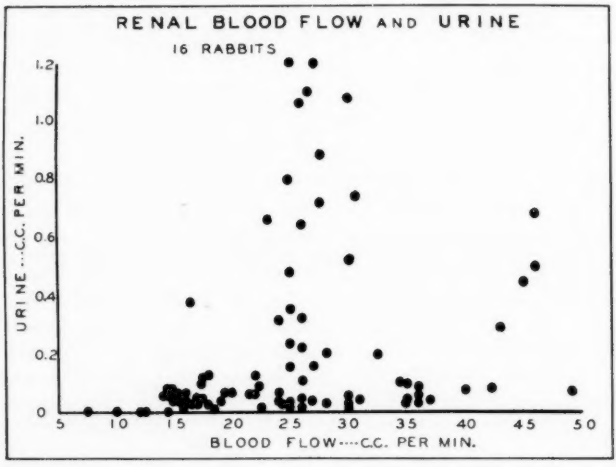

Fig. 4. Relation between renal blood flow and bladder urine in 16 rabbits

but 5 animals of each group, the amount of urine increasing in rabbits on the average from 0.06 to 0.62 and in dogs from 0.21 to 2.66 cc. per minute. The results of 4 typical experiments on each species are charted in figure 3 and of the entire series of rabbit experiments in figure 4. No consistent

parallelism between blood and urine flows enduring throughout an entire experiment was found in either species. Nos. 4 and 8 in figure 3 are examples of 3 experiments on rabbits and 4 experiments on dogs in which parallelism was most nearly complete. Successive periods could be seen in each experiment in which blood and urine flows varied in the same direction, but even this relationship was absent in 43 of 67 periods on rabbits and 80 of 124 periods on dogs where significant variations in urine volume occurred. The parallelism proved to be no better when variations in urine volume were between 0.02 and 0.10 cc. per minute in rabbits or 0.2 and 0.5 cc. per minute in dogs than when diuretic levels were reached (nos. 3 and 5, fig. 3). Considerable alterations in blood flow were also observed without parallel alterations in urine volume. The sole connection between urine and blood flows which has been consistently observed was a diminution or cessation of urine occurring when the blood flow was sharply lowered by the administration of adrenalin or salyrgan (no. 1, fig. 7), or by the approach of death. The minimum blood flow necessary for the formation of urine in rabbits appeared to be in the neighborhood of 1.5 cc. per gram of kidney per minute.

In table 1 appear the results of creatinine clearances measured during water diuresis in 8 rabbits and 12 dogs. Although all but one of the experiments on rabbits show an increase in the clearance to accompany the increase in urine volume, the increase is not proportional to the change in urine and is absent in the most marked diuresis. In the experiments on dogs the changes in clearance were often large but they were not consistently in one direction.

Comment. Since the experiments of Van Slyke and his collaborators (4) were not designed to measure renal blood flow during water diuresis, the only existing estimations of this character are those of Jannsen and Rein (5) and Handovsky and Samaan (6). Both authors used the Rein stromuhr but their results are contradictory, the former finding no alteration in blood flow with the diuresis, the latter finding an increase in blood flow to precede it. Both publications are available only in abstract but it may be remarked that one author worked with decerebrate dogs, the other with dogs which though described as "conscious" had cannulae in the carotid arteries and the ureters, and that in neither case was the stromuhr calibrated except on an artificial schema. Our experiments (fig. 3) agree with those of Jannsen and Rein in indicating that changes in blood flow cannot be instrumental in producing the diuresis.

Unchanged clearances of creatinine and non-metabolized sugars have been repeatedly described during water diuresis in dogs (7, 8) and are confirmed in these experiments (table 1). They have been interpreted to mean that the diuresis is not caused by an increase in glomerular filtrate. In rabbits, on the contrary, Kaplan and Smith (9) have found creatinine

TABLE 1

Changes in creatinine clearance during changes in urine volume

	RABBITS		DOGS	
	Urine change	Clearance change	Urine change	Clearance change
	Water diuresis			
	cc. per minute	*cc. per minute*	*cc. per minute*	*cc. per minute*
	0.00	+0.40	+0.17	−1.30
	+0.02	+1.20	+0.21	+4.00
	+0.08	+0.20	+0.27	+11.00
	+0.09	+1.90	+0.50	+3.80
	+0.10	0.00	+0.70	−2.00
	+0.11	+0.40	+1.04	−0.10
	+0.11	+0.30	+1.12	−2.30
	+0.16	+0.20	+1.18	+9.90
	+0.27	+0.10	+1.28	+2.70
	+0.30	+0.50	+1.40	−2.60
	+0.46	−0.30	+1.45	−2.60
			+1.65	−7.80
			+1.95	−8.70
			+2.26	−0.20
			+3.32	+1.20
			+5.66	+14.70
Av.	+0.15	+0.50	+1.51	+0.62
	Pituitrin antidiuresis			
	−0.47	−1.7	−0.90	+3.40
	−0.68	+0.2	−1.20	−2.20
	−0.90	+0.4	−1.55	+2.3
	−1.20	+0.1	−1.80	+12.5
			−1.80	−6.9
			−1.95	+7.2
			−2.10	+6.5
			−2.10	−1.5
			−2.15	−5.6
			−3.90	+0.3
			−5.70	−9.2
Av.	−0.81	−0.3	−2.29	+0.8
	Xanthine diuresis		**Mercurial diuresis**	
	+0.05	−0.5	+0.40	−1.9
	+0.08	+0.6	+0.45	−4.9
	+0.09	+2.4	+1.05	+7.0
	+0.20	+7.3	+1.70	+6.1
	+0.28	+3.2	+2.80	−0.6
	+0.32	+0.9	+3.30	−2.1
	+0.40	+0.2		
	+0.53	−0.1		
	+0.60	+1.3		
	+0.64	+0.1		
Av.	+0.32	+1.6	+1.62	+0.6

clearances to increase *pari passu* with urine volume and believe changes in glomerular filtrate to be an effective factor in the diuresis. The experiments of table 1 show changes in the same direction as theirs but so much less marked and consistent as scarcely to support their conclusion. If an increase in glomerular filtrate occurs, our results force the conclusion that it cannot be due, to an increase in blood flow.

PITUITRIN ANTIDIURESIS. *Results.* In the presence of a water diuresis, 0.2 cc. pituitrin was injected subcutaneously into 6 rabbits and 0.5 cc. subcutaneously into 14 dogs. Urine volumes fell sharply within 30 minutes, in rabbits from an average of 0.78 to 0.10 cc., in dogs from 2.32 to

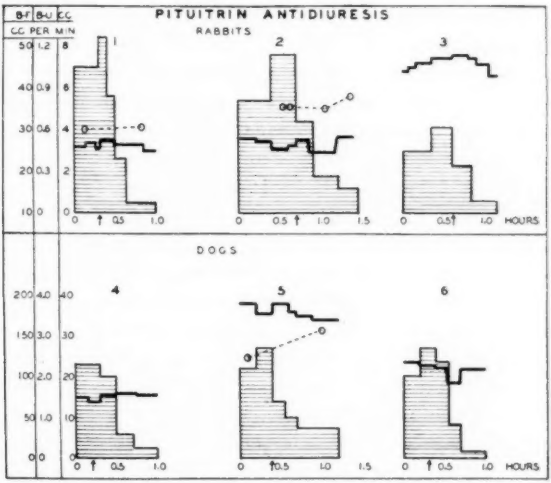

Fig. 5. Changes in renal blood flow (solid line, B.F.), bladder urine (shaded column, B.U.), and creatinine clearance (open circle, C.C.) produced by subcutaneous injection of pituitrin (arrow).

0.32 cc. per minute. Renal blood flow, observed during the development of the antidiuresis, fell somewhat in 16 of the 20 experiments but the variations were no greater than those existing during periods of constant urine flow, and in 19 experiments blood flow during some period of the antidiuresis was as high as or higher than it had been during the control period. The results of 3 typical experiments on each species appear in figure 5. No. 6 is the most suggestive example of a causal relationship between blood flow and antidiuresis which was encountered. In 3 experiments the blood flow showed an apparent marked increase following the injection of pituitrin.

The results of creatinine clearances, performed on 4 rabbits and 11 dogs,

appear in table 1 and show that no consistent reduction accompanied pituitrin antidiuresis in either animal.

Comment. Renal blood flow following the injection of pituitrin has been measured in dogs with the Rein stromuhr by Jannsen and Rein and by Handovsky and Samaan to whose experiments reference was made in the preceding section. Our results (fig. 5) agree with those of Jannsen and Rein, and with those obtained by Starling and Verney (10) on the isolated perfused dog's kidney, in indicating that a reduction in renal blood flow plays no part in the antidiuresis in either dog or rabbit.[5] The contrary result described by Handovsky and Samaan was presumably due to their intravenous injection of the drug. A decrease in blood flow can be produced by the intravenous injection of pituitrin but it is not essential to the production of antidiuresis.

The absence of a reduction in creatinine clearance (table 1) is consistent with the results which have been reported on dogs and on man (11, 13) and has been interpreted to mean that changes in glomerular filtrate are not the mechanism of the antidiuresis. This interpretation receives support from Gersh's experiments with injected ferrocyanide (14).

XANTHINE DIURESIS. *Results.* Twelve milligrams of Thephyldine, injected intravenously into each of 10 rabbits, produced diuresis which appeared within five minutes and usually lasted more than thirty minutes. In the control periods urine flow averaged 0.09 cc. per minute, at the height of the diuresis 0.37 cc. per minute. The preliminary injection of pituitrin in 5 animals did not alter the extent or duration of the diuresis. The results of 4 typical experiments are charted in figure 6. Directly after the injection, in 7 of the 10 experiments, there was an increase in blood flow of from 4 to 11 cc. per minute (nos. 1–3, fig. 6) but in every instance save one this increase was briefer than the diuresis, lasting an average of 9 as opposed to 30 minutes, and the blood flow returned to or below its former level while the diuresis continued. In one experiment the increase in blood flow remained below a level which had existed during low urine flows several hours previously. In 3 experiments little or no increase in blood flow accompanied the increase in urine (no. 4, fig. 6).

Creatinine clearances have been determined during these diureses on 8 rabbits with results which appear in table 1. There is a rise in the clearance in 8 out of 10 periods but it is not proportional to the extent of the diuresis and is either absent or very slight in 3 of the 4 largest diureses.

[5] In other animals a reduction in blood flow may occur; this has been assumed to be the case in birds and reptiles (11) and constriction of renal blood vessels has been seen in frogs by Adolph (12) and by observers in this laboratory. A reduction in size of the dog's kidney which was observed by x-ray during pituitrin antidiuresis (unpublished observations by A. E. Livingston and one of the authors in 1929) must have been due to alteration in tubule volume rather than to a reduction in the size of the vascular bed.

Comment. The statement of Jannsen and Rein (5) that renal blood flow increases following the oral administration of caffeine does not provide sufficient information as to the extent or duration of the increase to permit comparison with our experiments and there have been no other observations of renal blood flow during xanthine diuresis in unanesthetized animals. Our results however (fig. 6) are very similar to those obtained by Cushny and Lambie (15) who made interrupted readings on anesthetized eviscerated rabbits by the Barcroft and Brodie technique (3). We agree with their conclusion that changes in blood flow cannot be wholly responsible for the diuresis. But that changes in blood flow may play some part in the

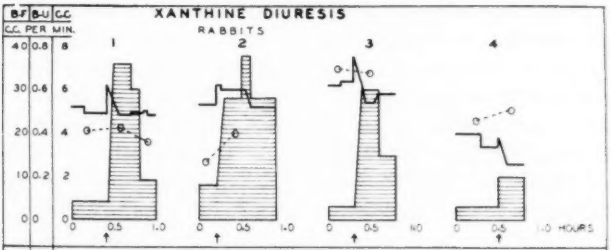

Fig. 6

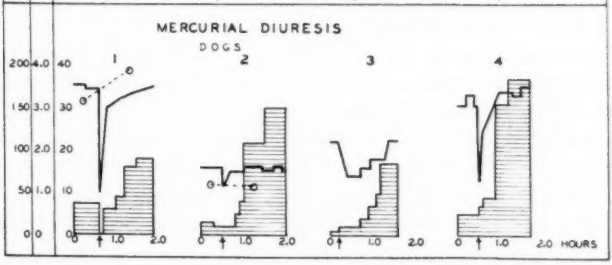

Fig. 7

Figs. 6, 7. Changes in renal blood flow (solid line, B.F.), bladder urine (shaded column, B.U.), and creatinine clearance (open circle, C.C.) produced by intravenous injection of Thephyldine and salyrgan (arrows).

diuresis cannot be denied since they usually occur, since the diuresis was notably less in the experiments where little or no increase in flow occurred (no. 4, fig. 6), and since the only animals which did not respond by a diuresis had blood flows of less than 2 cc. per gram of kidney per minute.

Creatinine clearances during xanthine diuresis have not been previously studied in rabbits but have been repeatedly investigated in dogs and man. The results have been conflicting; Schmitz (16), for example, using enormous doses of the diuretic, described a marked increase in clearance which Davenport et al. (17) and Blumgart et al. (18), using smaller doses, have been unable to demonstrate. Conclusions as to the part glomerular

filtrate plays in the diuresis have of course reflected this conflict. Our doses have been of the order of those employed by the latter authors, and our results (table 1) support them in making it impossible to conclude that alterations in glomerular filtrate are the cause of the diuresis. Our confidence in the accuracy of the clearance method, however, is not sufficient (see below) to make us deny that effective increases in filtrate may have occurred.

MERCURIAL DIURESIS. *Results.* One cubic centimeter of salyrgan, injected slowly by vein into each of 11 dogs, increased urine output from an average of 0.34 to 2.10 cc. per minute. The diuresis, preceded by a latent period averaging 28 minutes, usually continued unabated during the hour that observation was continued and was unaffected by the preliminary subcutaneous injection of pituitrin. The results of 4 typical experiments are charted in figure 7, where the most suggestive case of parallelism between blood and urine flows appears as no. 4. Within a minute of the injection there was a sudden marked reduction of blood flow in 8 of the 11 animals, but the flow usually returned close to its control level in the succeeding 15 minutes. The diuresis began and continued in 8 experiments while the blood flow was either below or at the control level and in two of the remaining three experiments (no. 4, fig. 7) the blood flow did not increase more than 7 cc. per minute beyond this point.

In 4 of the 6 experiments in which they were measured (table 1) the creatinine clearances were decreased during the period of the diuresis.

Comment. There is now sufficient evidence to prove that the diuretic action of mercury compounds is not extra-renal (19, 20). Renal blood flow has not previously been measured during the diuresis but the above results indicate that it can play no part in the increase of urine. The absence of consistent clearance increases in table 1 confirms similar previous findings (16, 17) which have been interpreted to mean that the diuresis is not due to an increase of glomerular filtrate but rather to a decrease in the amount of fluid reabsorbed by the tubules. This deduction is consistent with the known site of action of mercury salts as revealed in bichloride poisoning.

FURTHER OBSERVATIONS. *Magnitude of renal blood flow.* Renal blood flow has been observed during the control periods of these experiments in 14 rabbits with kidneys weighing from 6.6 to 9.3 grams. It varied from 1.5 to 4.8 and averaged 3.2 cc. per gram of kidney per minute. No measurements on unanesthetized rabbits have heretofore been made but in dogs Jannsen and Rein (21), using the Rein stromuhr, found an average blood flow of 2.6 and Van Slyke and his co-workers (4), using their urea method, 4.0 cc. per gram of normal kidney per minute. Eight dogs of the present series in which the blood flow measurements were most reliable had kidneys weighing from 43 to 63 grams and flows from 1.8 to 4.4 aver-

aging 2.8 cc. per gram of kidney per minute. Kidneys hypertrophied as the result of preliminary contralateral nephrectomy[6] showed an increase in blood flow proportional to the hypertrophy but not the increase in excess of this which Van Slyke described.

Percentage of blood plasma removed by glomeruli. If creatinine clearances be accepted as a measurement of glomerular filtrate, simultaneous measurements of creatinine clearances and renal blood flow provide a basis for calculating the percentage of blood plasma which is removed during its passage through the glomeruli. In 29 such measurements on 10 rabbits (data of fig. 8) the average percentage was 30.2. Excepting two animals, in one of which the percentage was consistently low (av. 17.8), in the other consistently high (av. 50.2), the variations were between 22.8 and 39.6 per cent. No change was found in this relationship after the administration of pituitrin and no consistent change after the administration of Thephyldine. There are no previous measurements of this sort in rabbits. But in dogs, Van Slyke and his co-workers (4) concluded that an average of 13 per cent of the total amount of urea in the blood is removed in its passage through the glomeruli; assuming a hematocrit of 50 per cent, this would mean filtration of 26 per cent of the plasma in their experiments.

Anesthetics. Four rabbits and 14 dogs were anesthetized with intraperitoneal injections of sodium pentobarbital in dosage of 30 and 36 mgm. per kilogram respectively. Observations of renal blood flow, continued only for the 10 to 30 minutes required to complete anesthesia, showed no reduction in 3 rabbits or 10 dogs. The contrasting results of Herrick et al. (22) were presumably due to their intravenous injection of the anesthetic. Only 1 of 8 dogs to which ether was administered by inhalation showed decreases in renal blood flow at the time third stage anesthesia was reached.

Discussion. The measurements of renal blood flow and of creatinine clearance are at one in failing to demonstrate changes which could be considered the cause of the observed alterations in urine volume. To this statement the diuresis produced by Thephyldine forms but a partial exception. Do these results justify the conclusion that alterations in glomerular activity are not responsible for the changes in urine volume under discussion?

As far as the blood flow measurements are concerned[7] there is evidence to indicate that renal blood flow varies directly with glomerular filtrate

[6] In 16 dogs preliminary nephrectomy was performed 2 to 23 (average 9.6) days previous to installation of the stromuhr. Increase in wet weight of the experimental kidney was apparent within two days and averaged 59 per cent, in the whole group; 5 dogs with renal hypertrophy of this order had higher creatinine clearances than 8 dogs with nonhypertrophied kidneys of similar weights (an average of 39 as opposed to 29 cc. per minute).

[7] There is ample evidence that in measuring total renal blood flow the blood supply of the glomeruli is being measured (23, 24).

(4, 25). If this be true, no effective changes in glomerular filtrate can have occurred, for the changes in urine volume would have required at the least an equal change in glomerular filtrate and five times[8] this change in renal blood flow. Increases of this order represent five to ten per cent of existing flows, are demonstrably detectable by the stromuhr and yet did not occur in the course of the experiments. This reasoning however overlooks the possibility that changes in glomerular filtrate may occur without any change in renal blood flow, as a result of alterations in the interglomerular distribution of blood or in the intraglomerular blood pressure. The absence of relationship between blood flow and creatinine clearance (fig. 8) does not disprove that the two may be directly related in individual ani-

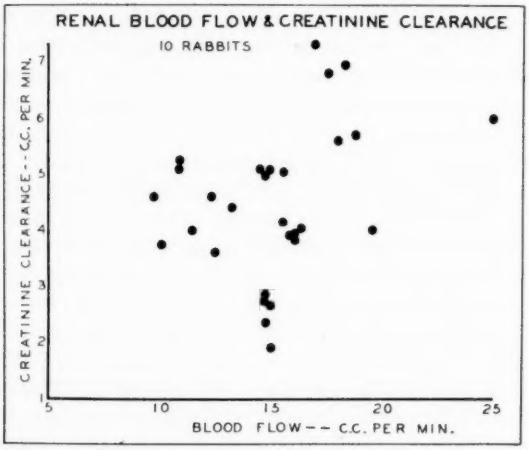

Fig. 8. Relation between renal blood flow (in terms of plasma) and creatinine clearance in 10 rabbits.

mals, but indicates that in a group of animals other factors are more important than blood flow in determining glomerular filtrate (26). If such factors (27) intervened in our experiments, changes in blood flow would not form a reliable criterion of changes in glomerular filtrate.

As far as creatinine clearances are concerned, their competence to measure changes in glomerular filtrate is open to question on two grounds: 1. The method depends on the assumption that no creatinine, or an unvarying percentage, is reabsorbed by the tubules. Pituitrin or diuretics may alter the reabsorption in such a manner as to invalidate the results.

[8] This figure is based on the average hematocrit of the experiments (60 per cent plasma) and requires that 30 per cent of plasma be filtered by the glomeruli (see above).

2. The variations which appear among the figures in table 1 and in similar investigations by other authors (11, 16) cast doubt on the accuracy of the method. It is possible to state that no gross consistent changes in clearance accompanied the changes in urine volume. But alterations in glomerular activity *could* be responsible for the urinary picture in the absence of such gross changes: if the tubules be assumed to reabsorb fluid in unaltered amounts, changes in glomerular filtrate of the order of 0.5 cc. in rabbits and 2.0 cc. per minute in dogs would suffice to explain the observed changes in urine volume. Clearly the clearance method is insufficiently accurate to discern changes of this degree. The probability of such an event must remain a matter of opinion.

Neither method, then, provides an unequivocal answer to our question. But in the failure of either one to indicate changes in glomerular filtrate, we find good evidence that the glomerulus is not responsible for the changes in urine volume.

SUMMARY

The renal blood flow of 16 unanesthetized rabbits and 20 unanesthetized dogs has been measured by a thermostromuhr during the changes in urine volume produced by water, by subcutaneous injections of pituitrin, and by intravenous injections of a xanthine and a mercurial diuretic. The sensitivity of the stromuhr was proved by calibration at the end of each experiment while it remained in its position on the blood vessel.

Changes in renal blood flow cannot be held responsible for the alteration in urine volume produced by water, by pituitrin or by salyrgan. A rise in blood flow occurred early in a majority of the diureses produced by Thephyldine but it was outlasted by the diuresis and cannot therefore be considered the sole mechanism responsible for it. Glomerular filtrate, as measured by creatinine clearances, was found substantially unchanged except for an inconstant increase during the diuresis produced by Thephyldine.

The objections to utilizing either renal blood flow or creatinine clearances as a measurement of glomerular function have been pointed out but, insofar as such measurements are acceptable, the results of our experiments indicate that alterations in tubule activity must be chiefly responsible for the observed changes in urine volume.

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METHODS OF COLLECTING FLUID FROM KNOWN REGIONS OF THE RENAL TUBULES OF AMPHIBIA AND OF PERFUSING THE LUMEN OF A SINGLE TUBULE[1]

A. N. RICHARDS AND ARTHUR M. WALKER

From the Laboratory of Pharmacology, University of Pennsylvania, Philadelphia

Received for publication August 25, 1936

The development of technique for microscopic observation of the amphibian kidney during life, for collecting fluid from single renal corpuscles and for determining quantitatively various urinary constituents in exceedingly minute amounts of fluid has resulted in the acquisition of information, of a sort not previously available, concerning the glomerular circulation and the composition of glomerular urine (1, 2, 3). Subsequent work has made it possible to subject the renal tubules of amphibia to similar direct study with the result that quantitative data of comparable quality can now be presented concerning the more important changes which the glomerular filtrate undergoes during its passage through the several parts of the uriniferous tubule. Fluid from different levels of the tubule has been collected and analyzed; the parts of the tubule which it had traversed have been identified; and, in special experiments designed to reveal the passage of substances through the tubule wall, the lumina of portions of single tubules have been perfused with artificial solutions.

In the papers which follow this the data thus far obtained will be presented; in this paper are described the manipulative and experimental procedures by which they were obtained.

The animals employed were adult Necturi (*N. maculosus*) and frogs (*R. pipiens*). The tubules in Necturus are of larger size and less tortuous course than those of the frog kidney.[2] Consequently the greater number of experiments has been made with Necturi.

NECTURUS. The induction and maintenance of anesthesia with urethane,

[1] The expenses of this work have been defrayed in large part from a grant by the Commonwealth Fund of New York. Brief descriptions of the technique have been presented before the American Physiological Society (This Journal **109**: 87, 107, 1934) and before the Harvey Society (Am. J. Med. Sci. **190**: 727, 1935).

[2] The kidney of an adult Necturus is about 6 times as large as that of a large frog (*pipiens*). The number of nephrons in one such Necturus kidney was 784; the average number in that of the frog is about 2000. The average capacity of a proximal convoluted tubule of Necturus (7 measurements) was 0.31 cu. mm.; in frogs, it was estimated to be about 0.05 cu. mm.

the exposure of the kidneys and their illumination by reflected light have been previously described (4, 5). Intravenous injections were made through a cannula inserted either into the anterior abdominal vein or a mesenteric vein. The ventral surface of the kidney was usually kept dry with cotton during an experiment to avoid the possibility of entrance of surface fluid into the tubule through its nephrostome. Urine from the ureter was collected either from an incision or by means of a cannula. Blood samples were taken from the posterior vena cava by puncture with finely pointed glass capillary pipettes containing dry heparin or potassium oxalate.

The anatomy of the kidney of Necturus has been well described by Chase (6). It seems desirable, however, to give a brief description of the disposition of the different parts of a nephron as they present themselves when the ventral surface of the kidney is studied with the binocular microscope (fig. 1).

The visible glomeruli are arranged in an irregular row parallel with and close to the mesial border of the kidney. The ciliated neck of the tubule and the ciliated tube which connects with the nephrostome on the surface of the kidney are rarely visible unless made so by an intracapsular injection of some opaque or colored material. The beginning of the proximal tubule is recognizable by reason of its large diameter, the pigmentation and apparent thickness of its wall. The first section of the proximal tubule, approximately one-quarter its total length, takes a relatively direct course toward the lateral border of the kidney. There it makes several convolutions, only fractions of which are visible; it ends as a short straight segment directed back toward the mesial border and is recognizable by the directness of its course, the transparency and relative lack of pigmentation of its wall. As it approaches the region of the glomeruli it narrows abruptly to form the intermediate tubule or narrow segment.

The narrow, ciliated intermediate tubule takes a relatively straight course towards the mesial border of the kidney but is rarely visible unless special procedures are adopted. At the level of or mesial to the glomeruli it becomes the distal tubule which remains narrow in its first part, convolutes several times in the immediate neighborhood of the glomeruli and usually lies too deep to be seen. The second segment of the distal tubule is wider and portions of it are often visible at the kidney surface; these are readily recognizable as small, highly transparent lacunae in the interstices of proximal tubules, blood vessels and connective tissue.

The collecting duct arises from the distal tubule about half way between the mesial and lateral borders of the kidney, runs a straight course towards the ureter, and is uniformly so deep within the kidney tissue as to be invisible.

The following figures are the averages of measurements by Dr. R. T.

Kempton[3] of the first four parts of the tubule in the kidneys, after dehydration, which have served in our experiments. In parentheses are given the number of measurements averaged. Neck, 0.9 mm. (135); proximal convoluted tubule, 13.9 mm. (133); intermediate tubule, 1.5 mm. (63); distal convoluted tubule, 8.1 mm. (65).

The precise site of puncture of the wall of a tubule with reference to the nephron as a whole can not usually be determined by simple inspection. Consequently in nearly all of the experiments, at the conclusion of a collection of tubule fluid, the pipette was filled with a 1:10 dilution of Higgins' india ink, reinserted into the puncture hole and the lumen of the entire nephron filled with ink. The kidney was then quickly excised and placed in absolute alcohol for dehydration, cleared in clove oil and an accurate scale drawing made of the injected nephron. The puncture hole was usually seen distinctly in the cleared preparation and measurements were

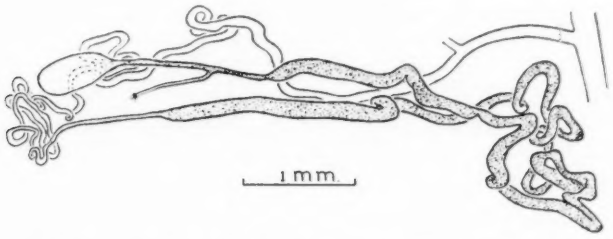

Fig. 1. A single nephron in the kidney of Necturus. From a scale drawing of an ink-injected cleared specimen. Stippled portions are the glomerulus, neck and proximal convoluted tubule.

made of the distances from it to the ends of the section of the tubule in which it occurred.

Collection of tubule fluid. The apparatus used was the mercury-filled, quartz capillary pipette system of Wearn and Richards (2). The tip of the pipette was sharply bevelled and about 20μ in diameter. The stand and micromanipulator were placed so that the pipette was in the same vertical plane as the section of tubule to be punctured and at an angle of about 30° with the surface of the kidney. The force required to push the point of the pipette through the peritoneum and tubule wall sometimes displaced the kidney to such an extent that when puncture suddenly occurred the wall of the tubule was torn or the tubule lying immediately beneath it was also punctured. Such an accident necessitated the selection of another tubule for the experiment.

For correct interpretation of analytical results it is essential that the

[3] A more complete account of the dimensional relationships of the Necturus tubule will be published by Dr. Kempton in the Journal of Morphology.

collected fluid shall not be contaminated with fluid drawn back into the pipette from a part of the tubule distal to the site of puncture. One precaution against this source of error was the practise of keeping the levelling bulb of the collecting system at such a height that the surface of the mercury in it was slightly higher than the tip of the collecting pipette in the lumen of the tubule. Greater certainty was secured by blocking the lumen of the tubule at a point immediately distal to the site of puncture. This was easily done when collection was to be made from the very beginning of the proximal tubule by inserting the pipette at the desired point and injecting a globule of mercury into the lumen: prevented from moving proximally by the narrowness of the tubule neck, it took a position just distal to the point of the pipette. Mercury, similarly injected, was sometimes used to block the distal end of the proximal tubule before collecting from its terminal segment; we came to regard this as an unnecessary precaution because, in a number of trials, we were unable to draw fluid

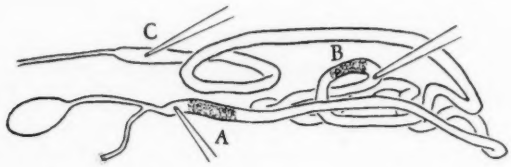

Fig. 2. Illustrating the insertion of a micropipette for the collection of tubule fluid from the beginning, *A*; the middle, *B*, and the distal end, *C*, of a proximal convoluted tubule of Necturus. Stippling at *A* represents a globule of mercury; at *B*, a column of colored oil.

back from the distal tubule through the narrow, ciliated intermediate tubule.

When fluid was to be collected from other parts of the proximal tubule a relatively thin mineral oil[4] was chosen as the obstructing fluid in preference to mercury because, owing to its lower surface tension, its injection through the capillary pipette could be controlled with far greater nicety. The injected column of oil moved with the current of tubule fluid to a position distal to the point of the pipette where it remained as long as all of the tubule fluid coming to the pipette was collected. It could be caused to move by slight change in the height of the levelling bulb of the collecting system: hence throughout a collection, it served as a sensitive indicator of the correctness of adjustment of the point of the pipette within the lumen of the tubule.

Collection of fluid from the intermediate tubule is difficult because its

[4] Atlantic Refining Company's "250 T" oil, saturated with Scharlach-R to increase its visibility.

lumen is narrow and it usually lies too deep to be easily visible. Its visibility can be increased by injecting a minute amount of diluted india ink into the distal end of the proximal tubule and allowing it to flow through the intermediate, or by injecting air and then mercury into the lumen of a distal tubule, thus forcing the air to move proximally through the intermediate tubule. The mercury blocks the distal end of the intermediate tubule, and the air in it is gradually absorbed. Then fluid can be collected in a pipette the tip of which has been thrust through the wall of the intermediate tubule.

Collection of fluid from the distal tubule is also difficult in that only a few small portions of its convolutions are visible at the ventral surface of the kidney. With practise they are easily distinguished by their location and appearance, but no estimate can be made of the site of puncture until the experiment has been finished, the tubule injected with ink and the map of the tubule drawn. For this reason it is largely a matter of chance whether or not the data accumulated in a number of experiments will show the progress of the changes which take place in fluid as it passes through the distal tubule.

Another difficulty arose because of the extreme ease with which fluid comes back into the pipette from the collecting duct and ureter. To avoid this the ureter was incised so that there was no impediment to escape of urine from it. The lumen of the tubule was blocked distally to the site of puncture by the injection of colored oil as described above.

Success in the collection of enough fluid for analysis from any part of the tubule obviously depends upon the delivery of an adequate amount of glomerular filtrate into the tubule. For this reason sluggish glomerular circulation, more frequently encountered in Necturi than in frogs, has, in our experience, required many animals to be discarded. For the same reason fluid may stagnate in the tubules and care must be taken that stagnant fluid be not included in tubule fluid collections. On occasion, also, tubule fluid from Necturi contains protein in concentrations from one-hundredth to one-tenth of that in blood plasma. The analyses however indicate that this circumstance is not associated with decrease in tubule function. Only rarely has either of these disadvantages been encountered in frogs.

Perfusion of the lumen of a proximal tubule. In order to answer questions concerning the passage of individual constituents of the urine through the wall of the tubule (reabsorption, secretion or diffusion) a procedure was developed in which, after the abolition of function of the glomerulus, an artificial solution, differing from normal glomerular urine in one or more particulars, was introduced into the lumen of the tubule at one point, collected for analysis at another. The procedure was as follows (fig. 3): Two capillary pipette systems were set up, one for perfusing, *P*, the other

for collecting, *C*. A nephron was chosen of which both ends of the proximal tubule were visible and accessible to puncture. One pipette, *P*, was inserted through the capsule of Bowman and mercury injected until the capsule, the neck of the tubule and the lumen of a short stretch of the proximal tubule were filled with mercury. (Pressure of the mercury remaining within the capsule stops glomerular filtration.) This pipette was then withdrawn, charged with the perfusion solution and its point inserted into that portion of the lumen of the proximal tubule which was filled with mercury. Upon forcing some of the solution to flow in, the mercury column in the tubule was broken and the distal fragment driven through the lumen to the distal end of the proximal tubule where it was arrested by the narrowness of the intermediate tubule. Injection of fluid from pipette *P* was stopped and the point of pipette *C* inserted into the lumen of the tubule at a point proximal to the mercury which blocked its distal end. (It was often necessary to remove pipette *P* during puncture with *C* to avoid tear of the wall at *P*. When this was the case, reinsertion of *P* was

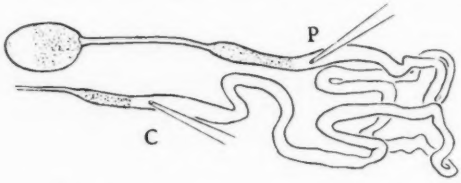

Fig. 3. Illustrating the insertion of micropipettes for perfusing the lumen of a single proximal convoluted tubule of Necturus. Stippling represents injected mercury.

made through the original puncture hole.) Perfusion from pipette *P* and collection in pipette *C* were then begun, the heights of the levelling bulbs of the two systems being so adjusted that the perfusion rate was similar to the normal rate of flow of glomerular fluid into the tubule. Both ends of the tubule being blocked, measurements of the amount of fluid which enters the tubule from pipette *P* and is collected in pipette *C* give information concerning the entrance or escape of fluid through the wall of the perfused tubule. By a slight modification of the technique the perfusion could be confined to any desired portion of the tubule.[5]

[5] In about half of the experiments of this type the collected perfusates contained easily detectable traces of protein, despite the facts that the original perfusion fluid contained none and glomerular fluid did not have access to the tubule. When such a perfusate was mixed with serum from a rabbit which had been immunized against Necturus serum, a precipitate formed, showing that in Necturus, under the conditions of these experiments, serum protein can find entrance into the lumen of the tubule through its wall (experiment by Dr. Chas. H. Hudson (7)).

Perfusion of the lumen of a distal tubule. The procedure just described could not be applied successfully to perfusion of the distal tubule because so much of it is invisible and inaccessible to puncture. Consequently a plan of perfusion via the ureter was adopted. A cannula was tied into the ureter and perfusion fluid introduced into it in such a way as to leave the tip filled with air. After connecting the cannula by rubber tubing with a reservoir containing the perfusion fluid the air in the cannula was forced back through the collecting ducts and tubules until it could be seen in the renal corpuscles or emerging from the nephrostomes. A capillary collecting pipette was then inserted into the distal end of a proximal tubule, a globule of mercury injected to prevent the descent of fluid from the glomerulus to the level of the point, and the perfusion reservoir raised to such a height as was necessary to provide a slow, constant flow of fluid through the distal and intermediate tubule into the collecting pipette.

In some experiments both the distal and the proximal tubule were perfused; in these the collecting pipette was inserted into the proximal end of the proximal tubule. In both instances the initial portion of the collection was rejected.

FROGS. *Preparation.* The brain was crushed with hemostatic forceps, the right kidney exposed and arranged for illumination with transmitted light in the manner described in previous papers from this laboratory. Urine samples were taken from the ureter; blood samples usually from the ventricle by puncture with capillary pipettes. The points of the quartz pipettes used in puncturing the tubules were commonly less than 10μ in diameter.

Collection of fluid from tubules. The frog's kidney possesses two advantages over that of Necturus; there are no nephrostomes through which fluid can gain direct access to the lumen of the tubule; the glomerular circulation is more vigorous so that the chance of collecting fluid which has been stagnant in glomeruli or tubules is absent. With respect, however, to ease of carrying out such procedures as those described above the frog's kidney is highly disadvantageous because of the much smaller size of the tubules and because of their tortuosity (fig. 4). The classical description by Nussbaum (8) of the disposition of the different parts of the tubule in the frog's kidney applies to the thicker portions of the kidney and not to the thinner part near the lateral border in which the more accessible tubules lie. Here the proximal and distal convolutions are not localized at the dorsal and ventral surfaces respectively. Both sections of the tubule are spread out in a thin sheet of tissue and direct inspection furnishes no clue as to the part of the tubule in which any visible loop is situated or the glomerulus from which any particular tubule arises. Most frequently the entire proximal tubule and the distal two-thirds of the distal tubule are more or less intertwined in the region lateral to the glomerulus; part of the first portion of the distal

tubule usually loops about the glomerulus to form a convolution mesial to it. For these reasons collection of fluid from predetermined levels of the tubule is exceedingly difficult. It is frequently possible to inject a dye solution or graphite suspension into the capsular space, watch it pass through the successive parts of the tubule and so to make rough identification of the relations of a visible segment to the entire tubule. Puncture of the wall of this segment can then be made and fluid collected with fair assurance of the approximate level of the tubule from which it came. In the majority of experiments however the plan has been adopted of selecting a distended segment of tubule at random and identifying its relations after

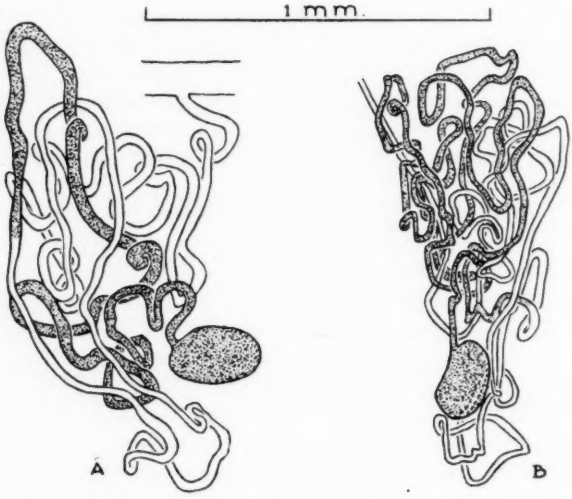

Fig. 4. Single nephrons in the lateral border of the frog's kidney. From a scale drawing of ink-injected cleared specimens. Stippled portions are the capsules and proximal convoluted segments.

collection from it was finished. This was done by filling the pipette with dilute india ink, reinserting it into the tubule and injecting the ink so slowly that the number and extent of convolutions through which it passed before it appeared in the glomerulus, in the first portion of the distal tubule or in the ureter could be observed. The accuracy of this procedure is only approximate, but, after experience had been gained, it was usually possible to be certain in which chief section of the tubule the puncture had been made and also in which third of that section. In some instances in which this method failed the punctured tubule was filled with india ink, the kidney excised and embedded in paraffin, and serial sections made. These revealed the site of puncture and showed whether it was in the proximal or

distal segment. The method of clearing the kidney and mapping the injected tubule which was effective in work with Necturus kidney was unsuccessful because the convolutions are so confusingly superimposed.

While the design of collecting fluid from various levels of the renal tubule in the frog can be accomplished by this method if the number of experiments is sufficient, the distal regions of the distal tubule are least apt to be included in a series of experiments by this chance method of approach. Consequently a special technique for identifying and puncturing this part of the tubule was devised. Oil colored with Scharlach-R was injected into the ureter through a cannula. Under the microscope it could be seen to fill the collecting ducts and to enter the terminal convolutions of some of the distal tubules. The location of these was marked for later identification by reference to fixed points in the field (e.g., blood vessels, pigment cells, other tubules), the ureteral pressure released and the tubules, collecting ducts and ureter allowed to be cleared of oil by the current of tubule fluid. The pipette was then inserted into one of the marked tubules and a short column of Scharlach-R oil injected. As soon as this had moved distally from the point of the pipette collection of fluid was begun. While blockage of the tubule distal to the point of puncture to prevent contamination of collected fluid is not believed to be as uniformly necessary as in Necturus the frequency with which reversed flow has been seen in the frog's tubule makes it an advisable part of the technique. It has the additional advantage of indicating the direction of flow within the tubule.

Perfusion of the lumen of the frog's tubule. The procedure is the same in principle as that described on p. 115. Mercury, however, can not be used to arrest glomerular filtration and to block the tubule because of the difficulty of injecting a sufficiently small amount. Oil is more easily managed; hence in preparing to perfuse a frog's tubule the perfusing pipette, charged with colored oil, was inserted into the intracapsular space and enough oil injected to fill the space and the lumen of about 0.5 mm. of proximal tubule. The pipette was then withdrawn, filled with the perfusion fluid, inserted into the oil-filled lumen of the proximal tubule and enough fluid injected to break the oil column and drive the distal fraction through the tubule to a position immediately distal to that judged to be favorable for the insertion of the collecting pipette. The injection was stopped during insertion of the collecting pipette and when this had been accomplished the perfusion was begun.

Perfect insertion of the two pipettes is more difficult in the frog than in Necturus because of the more compact arrangement and smaller dimensions of the tubule. Great caution is necessary here as well as in collections from normal tubules to avoid penetrating the dorsal wall of the tubule and entering the tubule which lies beneath it.

We make grateful acknowledgment to Dr. R. T. Kempton for the

drawings which illustrate this paper and for his valuable help in identifying sites of tubule puncture in the experiments described in the papers which follow this.

SUMMARY

Procedures have been described by which fluid, in amounts sufficient for analysis, can be collected from various identified levels of the renal tubules of Necturi and frogs. Methods are also described for perfusing different parts of the lumen of a single tubule with artificial solutions.

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THE TOTAL MOLECULAR CONCENTRATION AND THE CHLORIDE CONCENTRATION OF FLUID FROM DIFFERENT SEGMENTS OF THE RENAL TUBULE OF AMPHIBIA

The Site of Chloride Reabsorption[1]

ARTHUR M. WALKER, CHARLES L. HUDSON, THOMAS FINDLEY, Jr. AND A. N. RICHARDS

From the Laboratory of Pharmacology, University of Pennsylvania, Philadelphia

Received for publication August 25, 1936

The first applications of quantitative methods to the analytical study of glomerular urine from frogs and Necturi seemed to show that the Cl concentration (1) and the total molecular concentration (2) of this fluid are higher than those of the plasma from which it is derived. More recent work with improved technique has shown that those results were erroneous (3, 4, 5); that in both respects the composition of glomerular urine is that of a protein-free filtrate from plasma. Bladder urine of these amphibia is highly dilute and contains little or no Cl[2]; hence it is certain that selective reabsorption of solutes takes place in the tubules. In the experiments summarized in this paper, fluid collected from different levels of the tubules has been studied with results which lead to the conclusion that the reabsorptive processes, responsible for the dilution and the low Cl concentration of urine, are operative in the distal and not in the proximal tubule. This conclusion is opposed to that drawn by White and Schmitt (6) from experiments of different design.

Methods. The technique of collecting fluid from different levels of the tubules and identifying the site of collection has been described in the paper preceding this (7); the manipulative technique involved in the quantitative study of the minute amounts of fluid available has been described by Richards, Bordley and Walker (8). The volumes of fluid analyzed were from 0.1 to 0.5 c.mm.

[1] The expenses of this work were defrayed in large part from a grant by the Commonwealth Fund. A preliminary report of these experiments was made before the American Physiological Society in April 1934 (This Journal **109:** 107, 1934).

[2] Figures for the Cl concentration of bladder urine have not been included in the tables in this paper. During the course of the experiments 22 samples of Necturus urine were analyzed: in none was there more than 0.07 per cent NaCl; average, 0.024 per cent. Of 12 samples of frog's urine none contained more than 0.08 per cent NaCl; average, 0.04 per cent.

Total molecular concentrations of tubule fluid and plasma were compared by Barger's capillary tube method with slight modifications of its adaptation by White (2, 5) for comparison of plasma and glomerular urine. The figures which express the results show the number of micrometer scale divisions, each equivalent to 5μ, by which a column of tubule fluid, 2 mm. long, changed in length during 48 hours' equilibration in a capillary tube against heparinized blood plasma from the same animal.

Blood was taken at the beginning and at the end of each collection of tubule fluid. Usually the plasma of the first sample was compared with the tubule fluid. In other tubes the two plasma specimens were compared with each other and plasma was compared with bladder or ureteral urine; in addition a control tube was prepared in which all columns were of the same fluid. Significant difference between the two plasma specimens was rarely observed; significant change in the control tube, never.

The modification referred to above consisted in the use of a "water manipulator" (8) for introducing the fluids into the capillary tube whereby greater precision and uniformity were secured. The tubes were uniform in internal diameter (0.35 mm.) and the fluids were introduced in the following order: plasma, three columns, in length respectively, 8, 2 and 2 mm., tubule fluid, 2 mm.; plasma, 2, 2 and 8 mm. The air column between each two fluid columns was 1 mm. long. Measurements of the length of the column of tubule fluid and of the two adjacent plasma columns were made with a Zeiss filar micrometer 1 hour and 48 hours after the tubes had been prepared. During this time the tubes were kept in water at room temperature.

In control tests with known NaCl solutions the least difference in concentration which could be identified with certainty was 5 per cent: when the difference was greater than this the change in length of the central column was directly proportional to it. For example, in a series of 8 tubes the central column in each consisted of 0.57 per cent NaCl solution; the adjacent columns, 0.60 per cent: after 48 hours' equilibration the central columns had decreased in length by 2–3 scale divisions. In other similar series, in which the concentration of the central columns was less, the following figures were obtained:

Percentage difference in concentration	10	15	25	50	75
Decrease in central columns (scale divisions):					
Range	5–8	7–12	14–22	29–37	49–77
Average	6.6	9	17	34	59

There is reason to think that the accuracy of the method is less than this when applied to plasma and tubule fluid; acid production by blood cells before centrifugation, glycolysis in plasma and the presence of heparin in plasma, all would tend to increase the osmotic pressure of blood plasma.

Chlorides were determined by the ultramicro chromate method of Westfall (4). Results are expressed as NaCl. In about one-quarter of the experiments two blood samples were taken for analysis of plasma, one before the beginning, the other after the end of tubule fluid collection. The difference in Cl concentration of the two plasma specimens was as a rule

insignificant. In 18 instances both the total molecular and the Cl concentrations were determined in the same specimen of tubule fluid.

RESULTS. The comparisons of plasma and tubule fluid obtained in 121 experiments are graphically presented in figure 1: the analytical data of

TABLE 1

Comparisons of the molecular concentration of tubule urine with that of blood plasma from Necturi and frogs

EXPERIMENT NUMBER	SITE OF COLLECTION*	MOLECULAR CONCENTRATIONS	
		Tubule urine vs. plasma	Bladder urine vs. plasma
Necturus: Proximal tubule, proximal half			
		scale divisions	
2	1.4	+1	−64
8	2.9/11.6	+1	−56
12	1/3	−2	−80
Av. of 12.....		−1.6	−55 (10)
Necturus: Proximal tubule, distal half			
13	9.6/15.8	−6	−63
16	7.7/10.9	−4	−55
23	13.3/13.8	−9	−50
Av. of 14.....		−4.4	−56 (10)
Necturus: Intermediate tubule			
28	1/3	−4	−74
Av. of 2.....		−6	−77
Necturus: Distal tubule, proximal half			
31	3.2/7.1	−36	−81
33	1/2	−44	−74
Av. of 6†.....		−37.6	−70 (4)
Necturus: Distal tubule, distal half			
		scale divisions	
35	2/3	−29	−86
41	7.8/8.7	−66	−60
Av of 7.....		−45.5	−75
Frog: Proximal tubule			
2	1/4	−6	
4	1/2	−2	−75
7	3/4	−2	−48
Av. of 7.....		−4.6	−69 (5)
Frog: Distal tubule			
8	1.0	−25	−63
13	3/4	−67	−83
14	3/4	−55	−63
Av. of 10.....		−38	−69 (8)

* For explanation, see text.

† One determination (−6) omitted from average.

typical experiments together with averages of the entire series are given in tables 1 and 2.

The different designations of "Site of collection" in the tables indicate differences in method and accuracy of identification. Such a mixed

fraction, for example, as 2.9/11.6 in a section of the table headed "proximal tubule" means that the total length of the ink-injected proximal tubule in the cleared kidney was 11.6 mm. and that the puncture hole was found to be 2.9 mm. from the proximal end. A number with one decimal place

TABLE 2

Chloride concentrations in tubule urine and blood plasma from Necturi and Frogs

EXPERIMENT NUMBER	SITE OF COLLECTION*	NaCl IN		DIFFERENCE
		Plasma	Tubule urine	
Necturus: Proximal tubule, proximal half				
		per cent	*per cent*	*per cent*
1	0.9/10.7	0.42	0.45	+7.2
3	2.0	0.45	0.44	−2.2
13	2.9/11.6	0.48	0.48	0
16	4.5	0.41	0.44	+7.3
17	4.9	0.43	0.46	+7.0
Av. of 17				+6.5
Necturus: Proximal tubule, distal half				
19	10.2/16.2	0.35	0.38	+8.6
22	11.5/13.1	0.41	0.40	−2.4
27	End	0.40	0.43	+7.5
28	End	0.40	0.43	+7.5
Av. of 12, one (−20.5) omitted				+6.8
Necturus: Intermediate tubule				
30	1/3	0.43	0.46	+7.0
Av. of 3				+7.5
Necturus: Distal tubule, proximal half				
34	4.1/8.7	0.42	0.25	−40.5
Av. of 3, one (−6.4) omitted				−48.8
Necturus: Distal tubule, distal half				
37	2/3	0.48	0.33	−31.3
40	6.5/8.4	0.42	0.22	−47.6
42	9/10	0.46	0.12	−73.9
Av. of 8				−59.4
Frog: Proximal tubule				
1	1/4	0.53	0.51	−3.8
3	2/3	0.60	0.65	+8.3
7	4/5	0.49	0.56	+14.3
9	End	0.51	0.56	+9.8
13	End	0.56	0.59	+5.4
Av. of 14				+5.7
Frog: Distal tubule				
15	1/3	0.61	0.38	−37.7
17	1/2	0.50	0.27	−46.0
18	2/3	0.54	0.28	−48.1
Av. of 6				−50.9

* For explanation, see text.

gives the distance in millimeters from the proximal end of the section punctured to the site of the puncture and implies that it was impossible to measure the remaining fraction. When accurate measurement in the cleared kidney was impossible, the site of puncture was recorded as the

estimated fraction (1/3, 1/2, etc.) of the section of the tubule studied which had been traversed by the fluid before reaching the collecting pipette, the estimate being based upon measurements and sketches made during the course of the experiment.

Proximal convoluted tubule. In the total molecular concentration tests fluid collected from the first third of the proximal convoluted tubule changed so little in volume when equilibrated against plasma that its

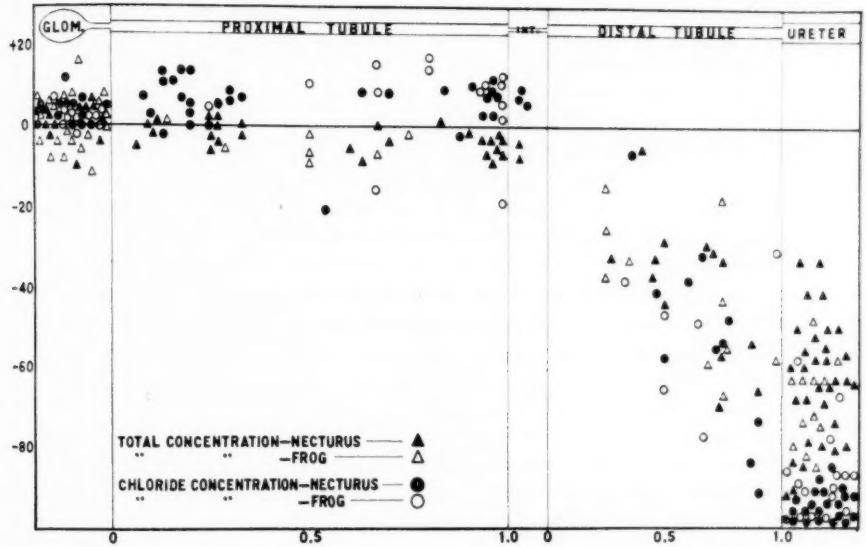

Fig. 1. Chart showing differences between blood plasma and fluid collected from various levels of the renal tubules with respect to total concentration and Cl concentration. Sites of collection of tubule fluid can be identified by the points on the abscissa which represent fractions of the total length of the 2 chief segments of the tubule. Zero on the ordinate represents plasma. For chlorides, figures above and below represent percentage differences from plasma; for total concentration, they represent the micrometer scale divisions (each 5μ) by which the 2 mm. capillary column of tubule fluid increased (+) or decreased (−) during 48 hours' equilibration against plasma. The representations of glomerular urine were taken from the previously published work of Walker (5) and Westfall, Findley and Richards (4).

concentration is regarded as the same as that of plasma. Fluid from the distal half decreased in volume slightly. The average of the changes in length of fluid columns in 14 experiments with Necturus was −4.4 scale divisions; in 7 experiments with frogs, −4.6 scale divisions; the greatest decrease was 9 scale divisions. Had these differences been obtained in control experiments with NaCl solutions the conclusion would be that on the average fluid from the distal half of the proximal tubule was about 8 per

cent less concentrated than plasma; that in 3 instances it was 15 per cent less concentrated.

The concentration of Cl in fluid from the proximal tubule appears from the figures in table 2 to be higher than that of plasma. In the experiments with Necturi the average of 17 analyses of fluid collected from the proximal half is 6.5 per cent higher than the average plasma value; of 11 analyses of fluid from the distal half, 6.8 per cent higher than plasma. In frogs, the average of 14 analyses of proximal tubule fluid was 5.7 per cent higher than the average Cl concentration of plasma.

It is apparent that there is a slight and unexpected disagreement between the two series of results, inasmuch as the total concentration of proximal tubule fluid appears to be somewhat less than that of plasma, while the concentration of chlorides, the chief factor in the total concentration, is somewhat higher. The discrepancy does not disappear when corrections are applied which take account of the presence of protein in the plasma (average in Necturus, 2.35 per cent according to White), of the effect of the Donnan phenomenon at the glomerular membrane on the composition of the glomerular filtrate, and of the reabsorption of glucose in the proximal tubule (9). Changes in plasma immediately after collection of blood and during 48 hours standing at room temperature in the total molecular concentration comparisons might conceivably be held responsible, but when glomerular urine was studied by the same methods such a difference was not encountered (5). In view of the technical difficulties of the experiments and the nature of the discrepancy we are inclined to attribute the disagreement to errors of technique rather than to the operation of active processes which lower the total concentration and raise the Cl concentration of the glomerular filtrate in its passage through the proximal tubule.

The intermediate tubule. Five determinations have been carried out on fluid collected from intermediate tubules of Necturi; two of total molecular concentration, three of Cl. The results are closely similar to those obtained with fluid from the distal end of the proximal tubule and give no indication of change in the fluid as it passes through this segment.

Distal convoluted tubule. Fluid collected from the distal tubule is much more dilute and contains much less Cl than plasma. In the experiments on total molecular concentration the average decrease in length of columns of fluid obtained in 5 experiments from the proximal half of the distal tubule of Necturus was 37.6 scale divisions, indicating that the concentration was less than half that of plasma; the average of the changes observed in 7 tests of fluid from the distal half of the distal tubule is −45.5 scale divisions, indicating a concentration about one-third that of plasma. In 10 experiments with frogs in which distal tubule fluid was tested the average decrease in volume was 38 scale divisions; the least, 14; the greatest, 67.

The average Cl concentration of fluid from the proximal half of the distal

tubule of Necturus is 48.8 per cent less than that of plasma (3 expts.); of fluid from the distal half, 59.4 per cent less (8 expts.). In the experiments with frogs the average of the Cl concentration of all of the samples of distal fluid is 50.9 per cent less than the average plasma Cl value.

The fact that fluid collected from the distal half of the distal tubule differs more widely from plasma than does that collected from the proximal half can only mean that the processes which result in these differences are operative in both portions. From these results we conclude that active reabsorption, resulting in the excretion of a dilute urine containing little or no Cl, proceeds throughout the length of the distal convoluted tubule.

In both groups of experiments it was found that the degree of dilution of fluid collected from near the end of the distal tubule was not as great as that of urine collected from the bladder or ureter. This may have been due to damage to the epithelium of the tubule resulting from puncture; it might also be explained by the selection for puncture of a tubule in which the volume and rate of flow of fluid were greater than in the majority of tubules in the kidney.

Repetition of the experiments of White and Schmitt. In their first studies of Necturus kidney White and Schmitt (6) undertook to determine the site of chloride reabsorption by observing at what level dog's red blood corpuscles, introduced into the lumen of the tubule, became laked. A 0.6 per cent NaCl suspension of the cells, stained with methylene blue to make them more easily visible, was injected into the renal corpuscle in amount sufficient to fill the lumen of the entire tubule. They could see that the wall of the proximal tubule became blue but, even with high magnification, were unable to discern intact corpuscles in its lumen. From this they concluded that the red corpuscles were "almost instantaneously" laked and implied that the dilution of the fluid responsible for laking was the result of extraordinarily rapid reabsorption of NaCl occurring in all parts of the proximal tubule. This conclusion differs so radically from that which we have drawn from our analytical results that repetition of their experiments was necessary.

In two minor respects we departed from the procedure of White and Schmitt; the dog's corpuscles were not colored with methylene blue, because, with our system of illumination, the unstained cells were easily visible within the tissue of the kidney: nor did we find it necessary to use higher magnifications in looking for corpuscles in the lumen of the tubule than in making the injections into the capsular space.

The following is a resumé of the results obtained with Necturi.

In two experiments in which there was little or no glomerular circulation the lumen of the capsule and of the whole proximal tubule was filled with a suspension of dog's red corpuscles in 0.6 per cent NaCl solution. An hour after the injection the corpuscles still remained in the tubule, unlaked.

In 7 experiments in which the glomerular circulation was active a suspension of dog's red cells in 0.7 per cent NaCl was injected by capsular puncture in volume sufficient to fill the intracapsular space and the lumen of the first two or three millimeters of the proximal convoluted tubule. The pipette was then withdrawn and the preparation watched closely while newly formed glomerular filtrate washed the injected cells through the length of the proximal tubule. In each case, in from 4 to 12 minutes after the injection, the corpuscles were seen moving through the lumen of the extreme distal end of the proximal tubule. In 3 of these experiments a pipette was inserted into the proximal tubule at its distal end as soon as possible after the corpuscles had appeared there, and fluid collected. It was transferred to a capillary tube and on examination was found to contain an abundance

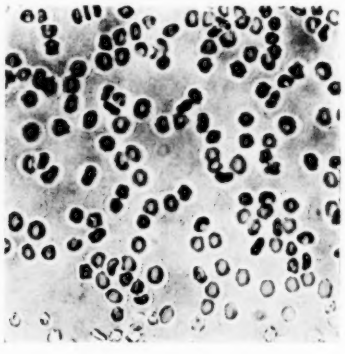

Fig. 2. Photomicrograph of fluid collected from the distal end of a proximal tubule of Necturus 12 minutes after injection of a suspension of dog's red blood cells into the capsule of Bowman from which the tubule originated. Because of the diameter of the tube in which the fluid was examined, relatively few of the cells were in good focus.

of unlaked corpuscles suspended in a colorless fluid. No hemolysis had occurred. Figure 2 is a photomicrograph of the fluid obtained in one of these experiments. During the interval between injection into the capsule and collection from the end of the proximal tubule the corpuscles were clearly seen moving through the lumen of the convolutions which were accessible to microscopic observation.

When the flow of fluid through the proximal tubule was relatively slow the corpuscles were seen to collect as stagnant sediments coating the dorsal aspects of the luminal surface, particularly of dependent loops and of the distal end of the proximal tubule, close to its transition into the intermediate tubule. When these sediments were stirred by stroking the surface of the kidney with a probe the corpuscles were seen to move distally with the current of tubule fluid.

In two experiments fluid was collected from the distal end of proximal tubules and mixed in a capillary tube with one-fifth of its volume of a suspension of dog's red cells. No hemolysis occurred in the succeeding 2 hours. In similar tests in which bladder urine or 0.2 per cent NaCl solution was used instead of tubule fluid hemolysis was complete in 2 minutes.

From these results we are compelled to conclude that the observations of White and Schmitt were erroneous.

SUMMARY

The total concentration and the Cl concentration of fluid collected from various levels of the renal tubules of Necturi and frogs have been determined in comparison with blood plasma from the same animals. The reabsorptive processes which are responsible for the high dilution and low chloride concentration of the urine of these amphibia are localized in the distal and not in the proximal convoluted tubule.

These results disagree with the conclusion of White and Schmitt that the site of selective reabsorption of Cl is the proximal convoluted tubule. Their observation that dog's red blood corpuscles, suspended in saline and introduced into the lumen of the proximal tubule, were laked there was not confirmed.

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THE REABSORPTION OF GLUCOSE FROM THE RENAL TUBULE IN AMPHIBIA AND THE ACTION OF PHLORHIZIN UPON IT[1]

ARTHUR M. WALKER AND CHARLES L. HUDSON

From the Laboratory of Pharmacology, University of Pennsylvania, Philadelphia

Received for publication August 25, 1936

The experiments reported in this paper concern the behavior of glucose in different parts of the tubule of the amphibian kidney and the influence of phlorhizin upon it. Previous work had shown that the reducing power of glomerular urine from frogs and Necturi is the same, within limits of experimental error, as that of the plasma from which it is derived (1). The same result was obtained in experiments with animals poisoned with phlorhizin. Firm basis was thus established for the inference that the absence of glucose from the urine of normal amphibia is the result of its selective reabsorption by the tubules and that phlorhizin glycosuria is the result of interference with this process.

Direct evidence that the proximal convoluted tubule is the site of glucose reabsorption in the normal kidney was offered by White and Schmitt in 1926 (2). Fluid, collected from proximal tubules of 4 Necturi, gave no reduction of Benedict's solution. Their experiments, however, were made in ignorance of certain technical pitfalls which the later work of White himself revealed (3); and since failure to avoid these pitfalls might well have led to the results they secured the soundness of their evidence is open to question. Our experiments, made with the advantages of that information, completely confirm the correctness of their conclusion. In addition they show that while the power of reabsorbing glucose is not restricted to any particular segment of the proximal tubule it is not possessed by the epithelium of the distal tubule. They also give direct information that the inference stated above concerning the action of phlorhizin is true.

METHODS. Necturi were anesthetized with urethane; in frogs, the brain was destroyed by a hemostat. The technique for collecting fluid from the tubules and for perfusing the lumen of a single tubule has been separately described (4).

Determinations of reducing power of tubule fluid, urine and blood plasma

[1] The expenses of this work were defrayed in large part from a grant by the Commonwealth Fund. A preliminary report of these experiments was made before the American Physiological Society in April 1934 (This Journal **109**: 107, 1934).

were made by the ultramicro-adaptation of Sumner's method (1). The results, including those of experiments with xylose, are expressed as glucose. Blood was collected shortly before the beginning and shortly after the end of a collection of tubule fluid: in Necturi, from the posterior cava; in frogs, from the ventricle of the heart. The average of these two specimens was compared with the glucose concentration of tubule fluid. In frogs the coeliaco-mesenteric artery and the portal vein were usually ligated to lessen the progressive increase in plasma glucose concentration which frequently occurs during an experiment.

RESULTS. *Reabsorption from the proximal convoluted tubule.* In 32 experiments with Necturi and 9 with frogs fluid collected from different levels of the proximal tubule has been analyzed. The experiments were made during the months of January to April, 1933 and 1934. In Necturi the average volume of tubule fluid collected was 0.29 c. mm.; the average duration of collection, 20 minutes. In frogs the average volume was 0.20 c. mm.; average duration, 38 minutes. When a sufficient amount of fluid was available, it was tested for protein with trichloroacetic acid. In the frog series, 4 tests were made, all with negative results. In Necturi, 9 tests were negative, 9 positive. No correlation was found between degrees of sugar reabsorption and presence or absence of protein. Plasma glucose concentrations in Necturi averaged 59 mgm. per cent (range, 33–105); in frogs, 53 mgm. per cent (range, 27–95). Urine collected from the ureter of Necturus at the end of an experiment usually contained reducing substance equivalent to a concentration of about 5 mgm. per cent glucose; bladder urine from the frog, reducing substance equivalent to 10 mgm. per cent of glucose.

In only 3 experiments with Necturi was the lumen of the tubule blocked (4) distally to the point of insertion of the pipette: in 4 of the 9 experiments with frogs, block was established. Because of this omission it might be objected that the collected fluid must have been contaminated with fluid sucked back from more distal segments. Several circumstances answer this objection. In the great majority of experiments the surface of the mercury in the levelling bulb of the collecting pipette system was kept at a height several millimeters above the surface of the kidney; as a rule the tubule was not collapsed during the collection. Had such contamination occurred to any considerable degree it seems impossible that the results could arrange themselves in the form of the curve which they actually give. The results of the experiments in which an oil block prevented any possibility of backward suction fit the curve of the rest of the results.

The circulation through the glomeruli of the nephrons from which tubule fluid was collected was consistently good. In a few experiments with Necturi the state of the venous and capillary circulation in the kidney was recorded as poor or sluggish. In these, somewhat contrary to expectation, there was no evidence of failure to reabsorb glucose.

The data of enough of the experiments to give a fair representation of the whole series are collected in table 1. Figure 1 shows graphically the percentage differences between the glucose concentrations of plasma and tubule fluid in all of the experiments.

Necturi. In 6 experiments, glomerular urine was collected and analyzed. The glucose concentrations differed from plasma by +9, −9, +5, +4, +3

TABLE 1

Glucose concentrations of blood plasma and fluid (T.F.) collected from different levels of the proximal tubules of Necturi and frogs

NUMBER	SITE OF COLLECTION	VOLUME OF T.F.	DURATION OF COLLECTION	PLASMA GLUCOSE			T.F. GLUCOSE	DIFF.
				I	II	Av.*		
				Necturi				
		c. mm.	*minutes*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*per cent*
5	1/16†	0.4	15	54	65	62	57	−8.1
7	1.7/17.0‡	0.57	14	84.5	97	93	81.5	−12.4
9	2.3/17.8	0.26	19	40.5	45	43	33.5	−22.1
10	1/8	0.15	22	40	42	41	22	−46.3
15	2.4/16.8	0.20	31	43	54	51	17	−66.7
18	1/4	0.18	35	55	57	56	31	−44.6
20	2.2/8.4	0.31	21	59.5	70	63.5	35	−44.9
24	3/4	0.21	20	60	64.5	63	5	−92.1
27	7/8	0.39	29	44	59.5	54	5	90.7
29	17.7/20.2	0.27	13	45	60.3	58	5	91.4
31	End	0.26	13	61	73.3	72	12	83.3
				Frogs				
39	1/8	0.18	30	58.5	67	64	50	−21.9
41	1/3	0.24	45	44	48	46	11	−76.1
43	2/3	0.25	42	24	28	26	0	−100

* The figures in this column represent the values, obtained by interpolation, which correspond to the mid-points of tubule fluid collections.

† This and similar fractions in this column represent approximate measurements of the fraction of the length of the proximal tubule between the beginning of the tubule and the point of puncture.

‡ In this and similar fractions in this column the denominator is the measured total length of the proximal tubule in millimeters; the numerator the measured distance from its beginning to the point of puncture.

and +1; average, +2 per cent. This confirms the results of previous work (1).

In 3 experiments fluid was collected as it flowed from the neck of the tubule into the proximal segment. The average of the differences between the glucose concentrations of these fluids and the plasma was zero. There

was no indication that the neck of the tubule plays a part in the reabsorption of glucose.

Ten collections, represented by experiments 5, 7, 9 and 10 in table 1 were taken from the proximal eighth of the proximal tubule. The average of the glucose concentrations of these was 35.6 per cent less than the average of the corresponding plasmas.

Seven collections (cf. expts. 15, 18, 20 of table 1) taken from levels of the tubule in the second eighth of its length contained glucose in concentrations which averaged 53 per cent less than the plasma.

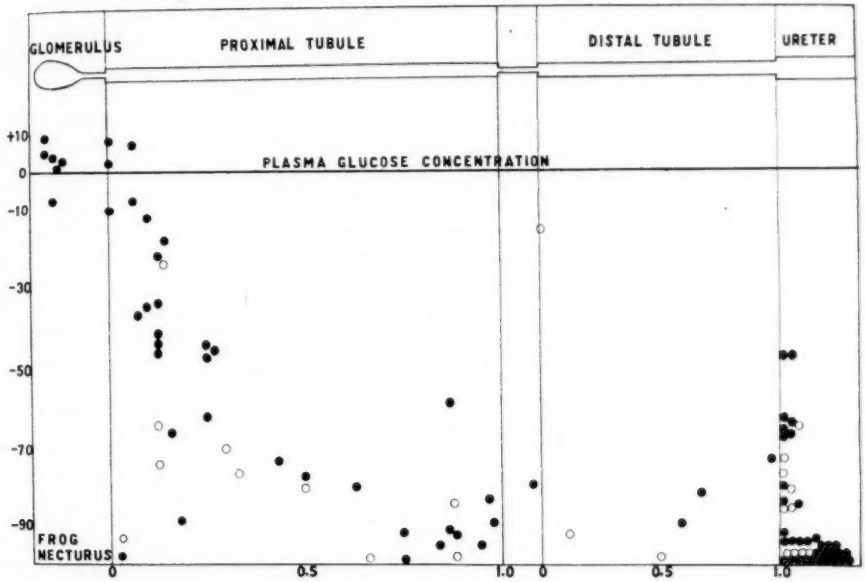

Fig. 1. Chart showing differences in glucose concentration between blood plasma and fluid collected from various levels of the renal tubules of normal Necturi and frogs. Sites of collection of tubule fluid can be identified by the points on the abscissa which represent fractions of the total length of the 2 chief segments of the tubule. Zero on the ordinate represents plasma, figures above and below zero represent percentage differences from plasma.

Twelve collections (cf. expts. 24, 27, 29, 31 of table 1) taken from various points between the end of the proximal quarter and the distal end of the proximal tubule gave glucose values ranging from 57 to 100 per cent lower than those of the plasmas, average, −85 per cent.

Frogs. In 9 experiments (cf. table 1, expts. 39, 41, 43), similar in plan to those with Necturi, a similar progressive decrease in the glucose concentration of the glomerular filtrate as it flows through the proximal tubule was demonstrated.

These results show with the greatest clarity that in both frogs and Necturi selective reabsorption of glucose from the glomerular filtrate occurs in the proximal tubule. They also show that under the circumstances of these experiments the greater part of the process is accomplished in the proximal half of the proximal tubule. Because of this fact the experiments do not show whether the distal half of the proximal tubule possesses the same power of reabsorption as the proximal half. To decide this question

TABLE 2

Reabsorption of glucose from Locke's solution perfused through the lumen of distal parts of the proximal tubules of Necturi

NUMBER	SEGMENT OF PROXIMAL TUBULE PERFUSED	VOLUME COLLECTED	TIME OF COLLECTION	GLUCOSE IN			DIFF. BETWEEN P.F. AND T.F.
				Pl.*	P.F.	T.F.	
		c. mm.	*minutes*	*mgm./ 100 cc.*	*mgm./ 100 cc.*	*mgm./ 100 cc.*	*per cent*
32	Distal 2/3	0.19	15	37	43	7.5	−82.3
31	Distal 1/2	0.90	30	31.5	48	10	−79.2
30	Distal 1/4	0.38	25	48	51	38	−25.5

* Pl. = plasma; P.F. = perfusion fluid; T.F. = collected perfusate.

TABLE 3

Comparisons of glucose reabsorption from Locke's solution perfused through the distal and the proximal convoluted tubules of Necturi

NUMBER	SEGMENTS OF TUBULE PERFUSED*	VOLUME COLLECTED	TIME OF COLLECTION	GLUCOSE IN			DIFF. BETWEEN P.F. AND T.F.
				Pl.	P.F.	T.F.	
		c. mm.	*minutes*	*mgm./ 100 cc.*	*mgm./ 100 cc.*	*mgm./ 100 cc.*	*per cent*
79	1. D + I	0.3	21	57	52.5	57	+6.7
	2. D + I + P	0.44	22	57	52.5	17	−67.6
82	1. D + I	0.65	15	50	49	47.5	−3.1
	2. D + I + P	0.5	20	44	49	5	−89.8
83	1. D + I	0.22	21	66	55.5	56.5	+1.8
	2. D + I + P	0.4	20	59	55.5	12	−78.4
84	1. D + I	0.25	23	71	52	52	0
	2. D + I + P	0.52	15	87	52	18	−65.4
	3. D + I	0.32	10	92	52	55.5	+6.7

* D = distal; I = intermediate; P = proximal tubule.

special experiments were made by the perfusion technique described on page 115 (4). In each the capsule of Bowman and a fraction of the proximal segment of a proximal tubule were filled with metallic mercury by capsular injection. Locke's solution containing 0.05 per cent of glucose was introduced into the lumen immediately distal to the mercury column and withdrawn from it by a pipette inserted at the extreme distal end of the proximal segment. The data of 3 experiments are given in table 2.

While the number of experiments is too small to allow quantitative comparison the results indicate that the capacity of reabsorbing glucose may be no less in the distal than in the proximal reaches of the proximal tubule. Certainly active glucose reabsorption is not restricted to a particular locality in the proximal tubule.

Reabsorption of glucose from the distal convoluted tubule. In 4 experiments with Necturi tubule fluid was collected from sections of the tubule distal to the proximal convoluted segment. In one the collecting pipette was inserted near the distal end of the intermediate tubule; in two others at points in the distal half of the distal tubule; and in a fourth the collection was made from a point about midway through a collecting duct. Three collections were made from analogous points in frogs' tubules. The analyses did not show greater completeness of reabsorption of glucose than did those of fluid collected from the distal parts of the proximal tubule. These experiments therefore are not competent to determine the question whether glucose can be returned to the blood during passage through the distal tubule. Hence the lumina of distal tubules were perfused with Locke's solution to determine whether its glucose concentration would be altered.

The Locke's solution was introduced into the tubules through a cannula in the ureter connected with a small perfusion bottle held at such a height above the kidney (about 13 cm.) that when a tubule was punctured with a collecting pipette the perfusion fluid would flow slowly from the ureter through the tubule to the site of puncture. Danger of contamination with fluid descending the tubule from the glomerulus was avoided by injecting a globule of mercury so that it obstructed the tubule proximally to the site of puncture. The data of 4 experiments are given in table 3. The concentration of glucose in fluid which had passed slowly through the distal and intermediate tubules only was the same, within experimental error, as that of the original perfusion fluid. But when, under the same conditions, in the same animal and in one case in the same tubule (no. 82), the fluid was allowed to traverse the proximal convoluted segment as well, the glucose concentration decreased by from 65 to 90 per cent. The results show that the distal convoluted tubule in Necturus does not share with the proximal the capacity of selectively removing glucose from the fluid in its lumen.

While no systematic attempts have been made to study the influences which may affect the process of glucose reabsorption, some of our experiments bear on this subject.

1. Dependence of degree of reabsorption on rate of flow through the tubule. The fluids analyzed in nine of the experiments represented by table 1 and figure 1 were collected from approximately the same level of the proximal tubules of Necturi, about $\frac{1}{8}$ of the distance along its length. If the assumption be made that rates of collection of tubule fluid were proportional to rates of flow through the tubule, the data of these experi-

ments (fig. 2) indicate that glucose reabsorption varies inversely with rate of flow.

2. Dependence of glucose reabsorption on the plasma glucose concentration. The experiments of G. A. Clark showed that in the perfused frog's kidney the reabsorption of glucose is limited by high concentrations of glucose in the fluid bathing the tubule (5). This observation is borne out by the results of a group of our experiments in which proximal tubules of Necturi were perfused throughout their entire length with Locke's solution containing 0.05 per cent glucose. In 18 experiments in which plasma glucose values ranged from 35 to 47 mgm. per cent the average reabsorption of glucose from the perfusion fluid was 69 per cent; in 18 experiments in which plasma glucose figures were from 51 to 70 mgm. per cent, average

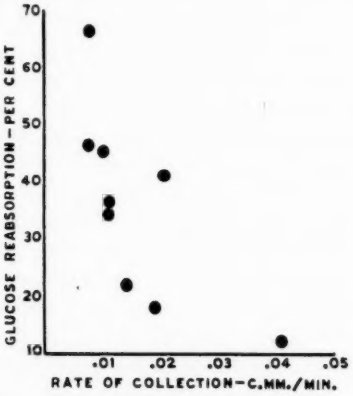

Fig. 2. Relationship between amount of glucose reabsorption and rate at which fluid was collected from the proximal tubules of Necturi.

reabsorption was 50 per cent; in 6 experiments in which plasma glucose was between 71 and 120, the average reabsorption was 26 per cent.

3. Glucose reabsorption against an osmotic gradient. In three experiments the lumen of a proximal tubule of Necturus was perfused with Locke's solution, modified to contain 1.0 per cent NaCl. Glucose was well reabsorbed in all three experiments (33, 74 and 100 per cent).

4. Influence of Ca and K. In one experiment the fluid with which the lumen of a proximal tubule was perfused contained no Ca or K; 50 per cent of glucose was reabsorbed.

Action of phlorhizin. A group of 39 experiments similar to those on normal animals has been made, 28 with Necturi and 11 with frogs, all poisoned with phlorhizin. Representative data are given in table 4 and all the results are charted in figure 3.

The dosage of phlorhizin was varied. It was never less than 30 mgm. per kilo and in the majority of experiments was from 150 to 550 mgm. per kilo. To Necturi phlorhizin was usually given intraperitoneally, 2 to 21 hours before the experiment. In frogs it was injected into the anterior lymph sac from 1 to 6½ hours before the beginning of the experiment. While it is impossible to say that maximal action of the poison was produced in any particular experiment the results give no evidence that the degree of action was affected by the variations in dosage used. The average urine/plasma glucose concentration ratio in Necturi was 2.54, in frogs 2.99.

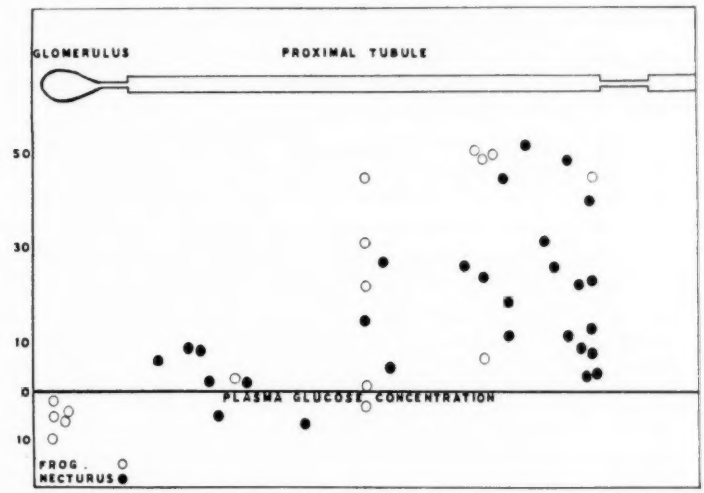

Fig. 3. Chart showing differences in glucose concentration between blood plasma and fluid collected from various levels of the proximal tubules of phlorhizinized Necturi and frogs. The position of the symbols relative to the diagrammatic tubule indicates the portion of the tubule from which fluid was collected; their position relative to the ordinate indicates the percentage by which the glucose concentration of the fluids differed from that of plasma.

The technique of preparation of the animals and puncture of the tubules was the same as in the experiments with normal animals. Blockage of the tubule distal to site of puncture (4) was effected in only 3 experiments with Necturi: the usual precaution of keeping the levelling bulb at such a height that the surface of the Hg was higher than the level of the kidney was uniformly observed. In all but 2 of the experiments with frogs the lumen of the tubule distal to the site of puncture was occluded by an injected column of oil. The rates at which fluid was obtained were of the same order as those in the experiments with normal animals. In Necturi, protein was detected both in the fluid collected from the tubules and in the

urine from the ureters more frequently than was the case with the normal animals. Tests were not made upon the tubule fluids collected from frogs.

In Necturi the average differences between tubule fluid and plasma were: for fluid collected from the proximal half of the proximal tubule (7 samples), +5.7 per cent: from the third quarter (5 samples), +19.2 per cent: from the last quarter or end (16 samples), +22.9 per cent. In frogs the average difference for 5 samples collected from the distal quarter or end of the proximal tubule was +40.3 per cent.

TABLE 4

Glucose concentrations of blood plasma and tubule fluid collected from different levels of the proximal tubules of phlorhizinized Necturi and frogs

NUMBER	PHLORHIZIN		SITE OF COLLECTION*	T.F.		GLUCOSE IN				DIFF.	GLUCOSE IN U.U.
						Plasma			T.F.		
	Dose per kilo	Time before experiment		Volume	Time	I	II	Av.			
						Necturi					
	mgm.	*hours*		*c. mm.*	*minutes*	*mgm./100 cc.*	*mgm./100 cc.*	*mgm./100 cc.*	*mgm./100 cc.*	*per cent*	*mgm. per cent*
3	424	5	1/8–1/6	0.15	18	82	85.5	84	91	+8.3	276
6	500	4	1/4	0.33	18	109	115	113	115	+1.8	210
9	45	18	5.9/10.9	0.48	32	165	184	175	222	+26.9	642
11	47	3½	5.4/7.6	0.45	52	70	78	74	93.5	+26.4	210
16	145	17	9.2/11.6	1.16	36	152	167	159	230	+44.7	376
20	169	16½	11.6/12.4	0.76	41	136	150	144	215	+49.3	420
22	400	16	End	0.30	13	131	144	139	171	+23.0	
26	127	16½	End	0.74	24	61.5	95.5	86	105	+22.1	172
						Frogs					
31	400	1½	1/2	0.085	35	52.5	46	48.5	59	+21.6	168
32	400	1⅙	1/2	0.26	42	38.5	39.5	39	47	+30.8	
38	400	2	3/4	0.24	40	49	49	49	74	+51.0	176
39	540	5	End	0.075	35	52	68	62	90	+45.2	

* See footnotes to table 1.

These results, considered in comparison with those obtained in normal animals, can only mean that the normal capacity of the epithelium of the proximal convoluted tubule to mediate the selective reabsorption of glucose is abolished by phlorhizin. The common belief, based on less direct evidence, is thus furnished with direct support.

The results also show, we believe, that in phlorhizinized amphibia reabsorption of water occurs from the proximal tubule. The increase in concentration of glucose in the glomerular filtrate as it passes through the

proximal tubule is not to be explained otherwise. The only alternative to this explanation is that under the influence of phlorhizin glucose is secreted by the tubule. This is regarded as untenable because of the strength of existing evidence (5, 6, 7) that the epithelium of the normal frog's tubule does not have the power to secrete glucose into the tubule; because of the difficulty of conceiving that phlorhizin can confer this power upon it *de novo*; and because of faults in the technique of older experiments (8, 9) which for a time led to the belief that phlorhizin glycosuria is the result of glucose secretion. In attempts to gain direct evidence on this point, the lumen of a single proximal tubule of a phlorhizinized Necturus was perfused with glucose-free Ringer's solution. Contrary to expectation the collected perfusate contained reducing substance. In four such experiments the concentrations (5–11 mgm. per cent) were no greater than is frequently found in bladder urine of normal (anesthetized) Necturi but in three others it was higher—about 50 per cent of the plasma glucose. That diffusion rather than secretion was responsible for entrance of glucose into the tubule in these latter experiments was indicated by tests for protein in the perfusates. It was absent from those in which glucose was lowest; present in considerable amounts in those in which glucose was high. A similar series of perfusions of proximal tubules of *normal* Necturi was made with like results. When little or no protein was detectable in the perfusate glucose was absent or present in low concentrations (5–18 mgm. per cent): when large amounts of protein were found, the glucose concentrations were as high as those encountered in the phlorhizin experiments. In none of either group did glucose in the perfusate reach the level of that in the plasma. Since it is inconceivable that serum protein is secreted into the tubule the parallelism between the glucose concentrations and the amount of protein in the perfusates can be taken as evidence that both substances entered the tubule by diffusion. The results of the experiments confirm the belief that neither the normal nor the phlorhizinized tubule secretes glucose.

The calculated amounts of water reabsorbed from the proximal tubule are not insignificant. To increase the glucose concentration of the glomerular filtrate by 23 per cent (average in Necturi) requires reabsorption of nearly one-fifth of its water: to increase it by 40 per cent (average in frogs) requires reabsorption of 29 per cent of the water. These figures represent about one-third of the total reabsorption of water from the renal tubule, calculated from average urine/plasma glucose concentration ratios in the 2 species.

Diffusion of sugars from the tubule. Having positive evidence that active reabsorption of glucose in the proximal tubule is abolished by the action of phlorhizin it was of interest to learn whether reabsorption by diffusion may still occur. For this purpose the lumina of proximal tubules

of phlorhizinized Necturi were perfused with Locke's solution, the glucose concentration of which was higher than that of the plasma. The salt concentration of the perfusion fluid was reduced so that its osmotic pressure should not be higher than that of the blood. Its composition was:—NaCl, 0.48; KCl, 0.01; $CaCl_2$, 0.02; $NaHCO_3$, 0.02 per cent. Glucose was added to make concentrations which, by analysis, ranged from 86 to 182 mgm. per cent. Measurements were not made of the volumes of fluid introduced and recovered; but in 3 experiments (nos. 40, 41 and 44) it was noted that the volume recovered was less than that introduced. Seven experiments were made with results which are shown in table 5.

With one exception (no. 42) the glucose concentrations of the collected perfusates were considerably less than those of the perfusion fluids. It may be significant that in this exceptional experiment the difference between the glucose concentrations of plasma and perfusion fluid (14 per cent) was

TABLE 5

Perfusion of proximal tubules of phlorhizinized Necturi with Locke's solution containing glucose in higher concentration than that of the blood

NUMBER	PHLORHIZIN		COLLECTION		GLUCOSE IN			DIFF. BETWEEN P.F. AND T.F.
	Dose	Time	Volume	Time	Pl.	P.F.	T.F.	
	mgm./k.	*hours*	*c. mm.*	*minutes*	*mgm./ 100 cc.*	*mgm./ 100 cc.*	*mgm./ 100 cc.*	*per cent*
40	51	2	0.21	31	41	89	53	−40.5
41	212	4½	0.23	13	48	86	71	−17.4
42	320	18	0.37	14	81	94	100	+6.4
43	159	3½	1.3	25	42	172	146	−15.1
44	53	2	0.5	30	49	160	113	−29.4
45	177	21	0.65	30	38	182	115	−36.8
46	171	4	0.2	17	57	86	72	−16.3

less than the others; and in the others there is some indication that the degree of difference between the plasma and perfusion fluid glucose influenced the degree by which the glucose concentration of the perfusion fluid was lowered in passing through the lumen of the proximal tubule. From these results the conclusion is drawn that glucose can diffuse out of the phlorhizinized proximal tubule of Necturus if its concentration in the tubule fluid attains a level sufficiently higher than that of the plasma: and from the results shown in table 4 it would appear that in Necturus this threshold difference is in the neighborhood of 23 per cent.

Reabsorption of xylose from the normal proximal tubule. At the time when the preceding experiments were made it was thought by some that neither secretion by the tubule nor reabsorption from it plays a part in the renal excretion of xylose in mammals and man, and that therefore its plasma clearance might be accepted as a measure of the volume of glomer-

ular filtration (10). The same belief was current concerning the renal excretion of glucose in phlorhizin poisoning. Having evidence that phlorhizin did not prevent diffusion of glucose from the tubule it was of interest to learn by similar methods whether in normal amphibia xylose could escape from the tubule through its wall. The proximal tubules of 11 Necturi and 3 frogs were perfused with fluid containing xylose in concentrations which ranged (by analysis) from 167 to 214 mgm. per cent: in 2 additional experiments the distal and intermediate tubules of Necturus were perfused with similar solutions from the ureter by the technique described on page 135. Typical results are shown in table 6.

Without exception the xylose concentration of the collected perfusates was less than that of the perfusion fluid. In Necturi the reducing power of the proximal tubule perfusates averaged 41.6 per cent less than that of the perfusion fluids (range, 11.0–73.7 per cent): in the 2 experiments in which

TABLE 6

Perfusion of proximal tubules of Necturi and frogs with Ringer's solution containing xylose

NUMBER	VOLUME OF FLUID		TIME	XYLOSE IN		GLUCOSE IN PL.	DIFF. BETWEEN P.F. AND T.F.	
	In	Out		P.F.	T.F.			
	c. mm.	*c.mm.*	*minutes*	*mgm./ 100 cc.*	*mgm./ 100 cc.*	*mgm./ 100 cc.*	*per cent*	
88		0.1	10	190	119	81	−37.4	Necturus
89	0.76	0.81	32	190	121	81	−36.3	Necturus
91	0.43	0.38	22	202	134	64	−33.7	Necturus
97	>0.29	0.29	20	183	88	41	−51.9	Necturus
99		0.24	34	194.5	135	105	−30.6	Frog
100	>0.08	0.08	30	175	110	82	−37.1	Frog
101		0.18	15	185	136	62.5	−26.5	Frog

the distal and intermediate segments only were perfused the lowering of reducing power was 12.6 and 28.5 per cent. The corresponding values obtained in perfusing the proximal tubules of frogs ranged from 26.5 to 37.1 per cent. Measurements of volume of fluid perfused and volume recovered showed that the decrease in concentration could not have been due to ingress of fluid from the outside. There can be no doubt that reabsorption of xylose occurred. Shannon (11) has recently published evidence which indicates that a fraction of xylose contained in the glomerular filtrate in dogs is actively reabsorbed from the tubules. The design of our experiments does not permit decision whether the escape of xylose from the proximal tubule in these amphibia is the result of an active process or of diffusion. Its reabsorption from the distal tubule must have been the result of diffusion.

Note on Lundsgaard's theory of phlorhizin action. In 1933 E. Lundsgaard (12) proposed that the explanation by Wilbrandt and Laszt (13) for the "active" absorption of glucose from the intestine, i.e., esterification in the cells of the absorbing membrane, could well be adopted for reabsorption of glucose from the renal tubule: and that phlorhizin, by inhibiting this process, prevents reabsorption and so induces glycosuria. His experiments, similar to those of Wilbrandt and Laszt with iodoacetic acid, showed that certain concentrations of phlorhizin in direct contact with the intestinal mucosa decreased the rate of absorption of glucose but not that of amino-acids. In digestion experiments with muscle-brei, muscle extracts, yeast and kidney extracts phlorhizin in concentrations of M/50 to M/200 was shown to inhibit the processes of esterification and dephosphorylation. He thought that in phlorhizin poisoning, comparable concentrations of phlorhizin might be found in the kidney, and hence that effects, comparable to those observed *in vitro*, might be produced there. Later experiments with the isolated mammalian kidney, however, in which the concentrations of phlorhizin in the kidney necessary to induce maximal action were calculated to be less than $\frac{1}{5}$ those required to produce the effects observed in the earlier experiments, seem to have induced him to abandon the theory, for he says (14) "it must be admitted that from these experiments no support can be gained for the assumption that the action of phlorhizin on the kidney is due to the earlier demonstrated effect of this poison on esterification."

During the course of the experiments described in this paper, actuated by thoughts similar to those of Lundsgaard, we have made experiments which, like his most recent ones, do not support his thesis. The experiments showed that (1) while phlorhizin in concentration of M/200 diminishes the rate of hydrolysis of glycerophosphate by kidney extracts *in vitro*, it does not stop it. When digestion was continued for 1, 2 and 3 hours (in Lundsgaard's experiments digestion periods were 10, 20 and 30 minutes) the diminution was inconsiderable (0–33 per cent).

(2) No consistent difference could be found between the phosphatase activities of extracts of kidneys from phlorhizinized rats as compared with those from normal rats.

(3) When iodoacetic acid was given to rabbits, rats and frogs in dosage of 0.1 mgm. per gram of body weight, the animals died without showing a detectable glycosuria.

(4) If the kidneys of frogs were perfused with a solution containing 1:5000 iodoacetic acid, they showed not only diminished ability to reabsorb glucose, but also a parallel failure of chloride reabsorption.

SUMMARY

As the glomerular filtrate flows through the renal tubule of normal Necturi and frogs its reducing power diminishes from an initial value equal

to that of the blood plasma to a value slightly above zero. The process of selective reabsorption of glucose which produces this change is localized in the proximal convoluted tubule: it is lacking in the distal convoluted tubule.

The degree of glucose reabsorption is lessened by increase in rate of flow through the tubule and by high concentrations of glucose in the blood plasma. The process is effective even when the osmotic pressure of the tubule fluid is doubled by increasing its concentration of NaCl.

In animals poisoned with phlorhizin the reducing power of the glomerular filtrate undergoes progressive increase as the fluid flows through the tubule. At the distal end of the proximal tubule in Necturi it is on the average about 25 per cent above that of plasma; in frogs, 40 per cent. At the distal end of the distal tubule in Necturi it averages $2\frac{1}{2}$ times that of the plasma; in frogs, about 3 times. It is obvious that active reabsorption of glucose by the proximal tubule is prevented by the action of phlorhizin. Evidence is offered in support of the view that the increase in reducing power is due to reabsorption of water. It indicates that on the average about one-third of the water reabsorption occurs in the proximal tubule; two-thirds in the distal.

Experiments on the phosphatase activity of phlorhizinized kidneys and on the action of iodoacetic acid on the kidney did not support the hypothesis of Lundsgaard that phlorhizin produces its characteristic effect by interference with esterification of glucose by phosphatase in tubule cells.

After phlorhizin has abolished active reabsorption of glucose by the tubule, passage of glucose into the blood may occur if the concentration in the tubule fluid is considerably higher than that in plasma. In normal animals xylose can pass from tubule fluid into the blood. In amphibia therefore neither the glucose clearance in phlorhizin poisoning nor xylose clearance in normal animals can furnish an accurate measure of the rate of glomerular filtration.

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THE SITE OF ACIDIFICATION OF THE URINE WITHIN THE RENAL TUBULE IN AMPHIBIA[1]

HUGH MONTGOMERY AND J. A. PIERCE

From the Laboratory of Pharmacology, University of Pennsylvania, Philadelphia, and the Harriet Lane Home, The Johns Hopkins Hospital, Baltimore

Received for publication August 25, 1936

Quantitative studies (1, 2) have confirmed the long accepted view, based upon earlier qualitative observations, that the pH of the glomerular filtrate in amphibia is the same as that of the plasma from which it is derived. In frogs an average value of 7.70 was found; in Necturi, two series of experiments gave averages of 7.54 and 7.39. The pH of frogs' urine in our experience has varied from 7.0 to 4.6; of Necturus urine, from 7.13 to 5.77. Acidification of the urine is exclusively a function of the tubules.

Experiments in this laboratory have indicated that the distal segment of the tubule rather than the proximal is the site of the process. Richards (3) observed that the color of a phenol red solution, introduced directly into the lumen of a frog's tubule, changed from red to yellow only when it came in contact with the epithelium of the distal third of the tubule. Ellinger and Hirt (4), however, from observations of the color of the contents of the tubules during the excretion of fluorescein, state that while the pH of glomerular fluid is 7.0 to 7.5 that of the fluid in the second section of the tubule in summer frogs is about 6.0. This change in reaction was not observed in winter frogs except after the intravenous injection of an acid salt (sodium bisulphate). Then the color of the epithelium of the proximal convoluted tubule indicated a pH of 5.0; that of the fluid in the lumen, less than 4.0. When the dye was injected into a frog whose renal arteries were ligated fluid with a pH of 5.0 to 4.5 was secreted into the lumen of the second section by the epithelium. Ellinger and Hirt conceive that the proximal tubule of the frog plays an important part in determining the reaction of urine; in summer, by reabsorbing alkali; in winter, if need arises, by secreting acid.

[1] The expenses of this work were defrayed in large part from a grant by the Commonwealth Fund to the University of Pennsylvania and from the John Howland Memorial Fund of the Johns Hopkins University. Doctor Pierce's collaboration was limited to the quantitative measurements with the microquinhydrone electrode. A preliminary report of these experiments was made before the American Physiological Society in April 1934 (This Journal **109**: 76, 1934).

In the experiments described in this paper quantitative measurements have been made of the pH of fluid collected from various levels of the tubule of Necturi with results which show that in this species the proximal tubule takes no significant part in the process of acidification. Colorimetric studies have been made which confirm belief that the same is true of at least one species of American frogs. Measurements were also made which identified the location and extent of the part of the distal tubule which possesses acidifying capacity and tests are described which partially reveal the intensity of this capacity.

METHODS. Adult Necturi (*N. maculosus*) and frogs (*R. pipiens*) were used; the former were anesthetized with urethane; frogs were decerebrated without hemorrhage by crushing the skull. The kidneys were exposed and illuminated in the usual way. The methods used in collecting tubule fluid and in perfusing fluids through the lumen of a single tubule have been described elsewhere (5).

Measurements of pH were made by two ultramicro-methods previously described by us, one of which is colorimetric (1); the other involves the use of a minute quinhydrone electrode (2). The former method was used for comparing tubule fluid with plasma; the latter for comparing tubule fluid with glomerular urine.

One particular item of technique merits especial consideration. Early in the work more than 20 experiments were made in which fluid, collected from the proximal tubules of Necturi, was found to be somewhat more alkaline than plasma. It seemed probable that this result was due to diffusion of CO_2 from the fluid within the lumina of the tubules. This thought was borne out by the observation that when a gentle current of CO_2 was directed against the surface of the kidney the acidity of the fluid within the lumen of the tubule was markedly increased. The procedure was then adopted of allowing oil, saturated with CO_2 at the average tension which obtains in Necturus plasma, to drip continually on the ventral surface of the kidney during the course of collection of a specimen of tubule fluid.[2] This precaution was observed in all of the experiments which are summarized in this paper.

RESULTS. *Quantitative measurements of the pH of tubule fluid from Necturus.* In table 1 are listed the results of 30 experiments arranged according to site of tubule puncture. The quinhydrone method was applied to 13 samples of fluid from the proximal tubule; to 6 from the intermediate and distal tubules; to 17 samples of ureteral or bladder urine. The colori-

[2] From a series of determinations of total CO_2 (Van Slyke and Neill) and pH (colorimetric) the calculated CO_2 tensions of Necturus plasma early in an experiment were found to be from 11.8 to 13.9, average 12.8 mm. Hg; late in an experiment they varied from 7.0 to 10.4, average 8.8. A CO_2 tension of 10.8 mm. was therefore chosen as that with which the oil was saturated.

TABLE 1

Quantitative measurements of pH of tubule fluid (T.F.), glomerular urine (G.U.) and bladder or ureteral urine (B.U., U.U.) from Necturi

EXPERIMENT NUMBER	SITE OF COLLECTION*	pH OF		DIFFERENCE	pH OF B.U. OR U.U.†	DIFFERENCE
		G.U.	T.F.			
Proximal tubule						
1	0.5	7.36	7.35	−0.01		
2	1.0	7.49	7.46	−0.03	7.05	−0.44
3	1.5	7.37	7.39	+0.02	6.35	−1.02
4	1.5	7.49	7.55	+0.06	7.13†	−0.36
5	2.5	7.33	7.32	−0.01	6.38	−0.95
6	5.0	7.21	7.22	+0.01	6.80†	−0.41
7‡	7.0	7.17	7.15	−0.02		
8	1/2	7.26	7.25	−0.01		
9‡	1/2	7.70	7.75	+0.05		
10	9.7/15.4	7.33	7.34	+0.01	6.40	−0.93
11‡	2/3	7.52	7.67	+0.15		
12	4/5	7.29	7.22	−0.07	6.79	−0.50
13	17.2/19.0	7.37	7.37	0		
14‡	14/15	7.66	7.70	+0.04		
15‡	14/15	7.52	7.54	+0.02		
16	End	7.59	7.59	0		
17	End	7.33	7.31	−0.02	6.66	−0.67
18	End	7.45	7.41	−0.04	5.77	−1.68
Intermediate tubule						
19		7.10	7.11	+0.01	6.88	−0.22
20‡	1/4	7.49	7.47	−0.02		
21‡	1.6/2.2	7.59	7.50	−0.09		
Distal tubule						
22	1.9/7.9	7.25	7.14	−0.11		
23‡	1.1	7.61	6.25	−1.36		
24	1/4	7.49	6.83	−0.56	6.36†	−1.13
25	4.3/8.5	7.64	7.57	−0.07	6.53	−1.11
26	3/4	7.12	6.63	−0.49	6.00	−1.12
27	3/4	7.57	7.34	−0.23	6.65†	−0.92
28		7.46			6.70†	−0.76
29		7.33			6.07	−1.26
30		7.33			6.03†	−1.30

* A single number in this column represents the distance in millimeters from the beginning of the segment to the site of puncture. A simple fraction represents an estimate of the length of the segment between its beginning and the site of puncture. A compound fraction is made up of measurements of the length of the segment proximal to the site of puncture (numerator) and the total length of the segment (denominator).

† Ureteral urine.

‡ In this experiment the pH was determined colorimetrically. The value in the G.U. column is the pH of plasma, corrected for the protein error.

metric method was applied to 5 collections from proximal and 3 from intermediate and distal tubules.

The pH values of 11 samples of proximal tubule fluid differ from those of the corresponding plasmas or glomerular urines by no more than 0.02 of a pH unit. While those of the remaining 7 differ more widely, when the signs of the differences are taken into account, the mean difference between proximal tubule fluid and plasma or glomerular urine is +0.008 pH unit.

From these results we must conclude that in Necturus no significant change in pH occurs during the progress of the glomerular filtrate through the proximal tubule. That this conclusion can be extended also to the short, intermediate segment is indicated by the three results obtained with fluid from it.

All of the pH values of fluid from the distal tubules are significantly lower than those of plasma, four impressively so. They therefore bear out the necessary corollary of the above conclusion that the distal tubule alone is the site of acidification. The fact that the differences are not as great as those between ureteral urine and plasma may be explained by the likelihood of choice of a tubule for puncture in which flow of fluid may be more rapid than in the majority, for it is certain that duration of contact of fluid with the acidifying cells has an important influence on the degree of acidity developed.

Change in reaction of fluid within the tubule as shown by indicator dyes. For adventitious reasons quantitative experiments of the type summarized above have not been made with frogs. Observations of the color of phenol red and of other indicators when they are present in the tubule fluid, however, leave no doubt that the conclusions drawn from study of Necturi apply also to frogs. We have repeatedly confirmed the observation in frogs and extended it to Necturi that when phenol red is added to the glomerular filtrate by intracapsular injection its color does not change from red (7.4 or more) to yellow (7.0 or less) until after the fluid containing it has entered the distal tubule (3). The fluid in the proximal tubule remains red even though, by appropriate obstruction, it is made to stagnate in the lumen for many minutes. On the other hand, in both species, the color changes to lemon yellow as soon as the fluid reaches a certain segment of the distal tubule, provided the flow of fluid in the tubule is not too rapid. The same result is obtained when the dye is injected intravenously or subcutaneously: all visible segments of tubules which can be identified as proximal contain red fluid; yellow fluid is seen only in the distal convolutions, collecting ducts and ureter.

The color change is abrupt: the extent of intermediate color (orange) is often no greater than 2 or 3 times the diameter of the tubule. The rapidity with which the color change occurs depends upon the rate of flow through the tubule as well as upon the buffering power of the fluid. When a

fraction of a cubic millimeter of a 0.1 to 0.5 per cent solution of phenol red in 0.6 per cent NaCl is injected rapidly into the lumen of a frog's tubule and the injection then stopped or slowed to the normal rate of tubule fluid flow, the change occurs in a few seconds; when the dye is similarly injected into a tubule of Necturus, the change is slower but is complete in less than 1

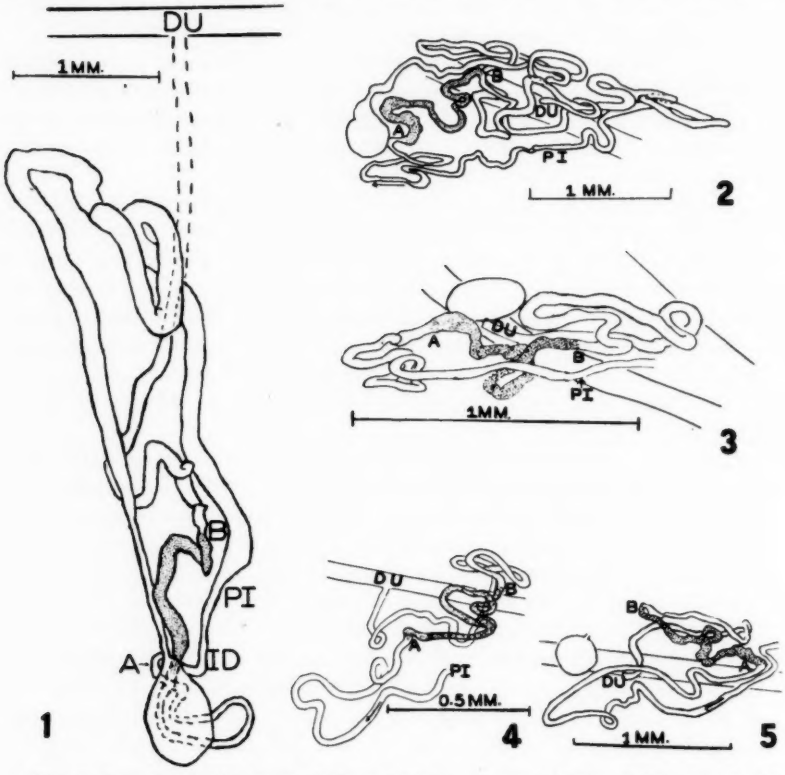

Fig. 1. Location and extent of the acidifying segment of the distal convoluted tubule. 1, an entire nephron of Necturus; 2, of frog. 3–5, distal tubules of frogs. *A* represents the acid change point in each; *A-B* (stippled) the entire acidifying segment. *PI* = junction of proximal and intermediate segments; *ID* = junction of intermediate and distal segments; *DU* = junction of distal tubule with ureter.

minute. The rate of injection may be so excessive that the color change does not occur.

The location of the acid change point in the distal tubule. In the following experiments perfusion of the kidney was adopted in order to insure sufficient visibility of the individual tubule studied. The perfusion fluid con-

tained chlorides and phosphates according to Barkan, Broemser and Hahn (6) and $NaHCO_3$ to make 0.1 per cent; in some experiments glycine to make 0.025 per cent was added; the fluid was saturated with oxygen containing 1.5 per cent CO_2, the resulting pH being 7.4–7.6. Aortic perfusion pressure was 20 cm.; renal portal, 8 cm. The rate of production of glomerular filtrate, as judged by the rate at which injected dye was washed down the tubule, was approximately the same as that in animals with intact blood supply. The description, given above, of the color changes in a kidney with normal blood supply is completely applicable to the perfused kidney.

The measurements given below are taken from scale drawings of individual tubules made on paper ruled in squares to correspond with the rulings of the eyepiece micrometer. Working with Necturus it was relatively easy to map an entire nephron; with frogs it was far more difficult and in the majority of experiments it was necessary to be content with a map of the distal and intermediate segments together with portions of proximal. One or two injections of the dye into the capsule or the lumen of the tubule were sufficient to identify the acid change point; as many more injections were made as were necessary to complete the drawing. Figure 1 contains reproductions of one such drawing of a Necturus tubule and several of frogs' in which the point is marked at which the color of phenol red began to change from red to orange.

Each of the following ratios represents a single experiment: the first figure is the measurement in millimeters of the distance from the proximal end of a distal tubule to the point at which change of color began; the second is the total length in millimeters of the distal convoluted tubule.

Necturi: 3.7/9.4 = 0.39; 2.3/6.9 = 0.33; 3.7/7.8 = 0.48; 2.8/7.3 = 0.38; 3.0/8.7 = 0.35; 4.1/9.9 = 0.41. Av. = 3.3/8.3 = 0.39

Frogs: 4.2/7.9 = 0.53; 3.8/7.7 = 0.49; 4.2/9.0 = 0.47; 4.5/9.4 = 0.48; 6.0/10.3 = 0.58; 4.2/8.3 = 0.51; 3.4/7.9 = 0.43; 2.2/5.7 = 0.39; 1.8/6.6 = 0.27; 4.8/8.8 = 0.43. Av. = 3.9/8.2 = 0.47

In most of the Necturi and in more than half of the frogs with which we have worked the acid change point coincides closely with an abrupt widening of the lumen of the tubule. This is illustrated in the drawings of figure 1. Histological examinations made by Dr. R. T. Kempton failed to reveal microscopic characteristics of the cells in the acid change area which distinguish them from those of other parts of the distal tubule.

The length of the acidifying segment of distal tubule. If a solution of phenol red is injected into the lumen of a tubule in such volume that the entire distal tubule is filled and so rapidly that acidification does not occur during the time of injection, and if then the fluid be kept from further movement in the lumen either by lowering the aortic perfusion pressure or by depositing a globule of mercury or a bubble of air in the lumen at the site of in-

jection, only that portion of the solution which remains in contact with cells possessing acidifying power becomes yellow. The lumen of the distal tubule takes on the appearance of a colored thread, both ends of which are red, the middle yellow. This identification of the acidifying segment

TABLE 2

Distances in millimeters 1, from the beginning of the distal tubule to the acid change point; 2, length of the acidifying segment; 3, from the distal end of the acidifying segment to the distal end of the distal tubule

	EXPERIMENT NUMBER		
	1	2	3
	Necturi		
1	5.1	1.3	3.3
2	4.6	1.6	4.9
3	2.3	2.3	6.0
4	3.0	2.0	
5	3.7	1.4	1.2
6	6.0	3.0	3.0
7	5.1	1.3	2.8
8	2.5	2.8	4.1
9	5.1	0.9	2.3
10	3.9	1.6	
Average.....	4.1	1.8	3.45
	Frogs		
11	3.8	0.4	
12	3.4	1.6	
13	4.2	1.5	3.3
14	4.5	2.1	3.0
15	6.0	1.1	3.2
16	2.9	0.9	
17	4.2	1.9	2.2
18	3.4	1.5	3.0
19	2.2	1.1	2.4
20	4.8	1.8	2.2
Average.....	3.9	1.4	2.8

enabled us, by scale drawing (fig. 1), to measure its length and to relate this to the length of the entire distal tubule.

Table 2 contains the results of such experiments. The figures in column 1 are the distances from the proximal end of the distal tubule to the acid change point; column 2, the length of the acidifying segment; column 3, the length of the remainder of the distal tubule.

From these measurements we can say that in Necturi and in frogs, on the average, the segment of the distal tubule whose cells accomplish the acidification of urine constitutes slightly less than one-fifth of the total length of the distal convolution and is situated somewhat nearer the distal than the proximal end.

The capacity of the tubule to acidify buffer solutions. A few experiments were made in the manner described in the section immediately preceding this in which sodium phosphate solutions of known buffer value, far greater than that of glomerular urine, were introduced into the lumen of distal tubules of frogs and the time noted for the change in reaction to occur.

A 0.33 M sodium phosphate solution, pH 7.5, containing 0.325 per cent of phenol red, held in contact with the acidifying cells by blockage of the tubule with mercury, became yellow in 60 seconds. A similar solution of phenol red in 0.6 per cent NaCl introduced into the same tubule changed color in 2 seconds.

In another tubule 0.1 M sodium phosphate, pH 7.5, containing 0.2 per cent phenol red became yellow in 30 seconds.

Effect of change in pH of the fluid outside the tubule upon the acidification process within it. Several experiments with frogs were made in some of which the pH of the blood was raised to 8.0 by the subcutaneous injection of large doses of $NaHCO_3$: in others, the kidneys were perfused with Barkan, Broemser and Hahn's solution to which no bicarbonate had been added, the pH of which was 6.2. In the former, cresol red (pH range 8.0–7.6) was introduced as the indicator of acidification of the tubule fluid; in the latter, chlorphenol red (pH range, 5.9–5.3). In both types of experiments the change in color of the tubule fluid from red to yellow occurred at the usual point in the distal tubule.

DISCUSSION AND SUMMARY

It is clear from the quantitative data given in the first section of this paper that acidification of urine in the tubule of Necturus is a function of the distal, not of the proximal segment of the tubule. Qualitative experiments show with equal convincingness that this is true of frogs (*R. pipiens*). None of our experiments confirms the belief of Ellinger and Hirt that the proximal tubule is concerned in the acidification process. We have seen nothing which indicates secretion of an acid fluid by the proximal tubule. In the experiments made by Bensley and Steen (7) comparable to the arterial ligation experiments of Ellinger and Hirt the fluid which was seen in the lumina of proximal tubules following the intravenous injection of phenol red was *red*.

The cells of only about one-fifth of the extent of the distal tubule possess the power of acidification and these are situated somewhat nearer to the distal than to the proximal end of the distal convolution. In many in-

stances the site of these cells was recognizable by a widening of the lumen which they inclose.

The demonstration that the acidifying cells can change the reaction of 0.33 M sodium phosphate from 7.5 to 6.8 in 1 minute furnishes an intimation that the functional capacity of these cells is far greater than is required to effect acidification of the normal tubule fluid. This solution has more than 100 times the buffer value of glomerular urine. The change described is equivalent to that which would follow the addition of one-fourth volume of 0.33 N HCl to 1 volume of the buffer solution.

Indications were found that the function of the acidifying cells persists after doses of bicarbonate sufficient to produce marked rise in blood pH. There were no indications that cells other than those normally involved assume a power of acidification when the pH of fluid which bathes them is excessively low.

None of the results obtained provides proof of the nature of the acidification process. The facts that the concentration of $NaHCO_3$ in urine approaches zero; that Cl is rarely present in more than a trace; that the concentration of urinary phosphates is only two to three times that of the plasma, that in turn being very low; all make it easier to believe that acidification is accomplished by reabsorption of bicarbonate than by secretion of acid (8). In the absence of conclusive evidence to the contrary, however, we are unable to deny that the acidifying cells may secrete acid; if they do, the salt resulting from its neutralization must be absorbed.

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THE RÔLE OF THE TUBULE IN THE EXCRETION OF UREA BY THE AMPHIBIAN KIDNEY[1]

With an Improved Technique for the Ultramicro Determination of Urea Nitrogen

ARTHUR M. WALKER and CHARLES L. HUDSON

From the Laboratory of Pharmacology, University of Pennsylvania, Philadelphia

Received for publication August 25, 1936

Urea is one of the substances which, in Amphibia, are more highly concentrated in the urine than in the blood plasma. Since its concentration in the glomerular urine is the same as that in blood (1), tubular processes exclusively are responsible for this increase. The primary purpose of these experiments was to identify the parts of the tubules of Necturi and of frogs in which these processes operate. It was hoped also that information might be gained concerning their nature.

Methods. Fluid was collected from single nephrons (2) and its urea concentration compared with the average of specimens of blood plasma taken by venepuncture (Necturus) or cardiac puncture (frog) at the beginning and end of each experiment. In more than four-fifths of the experiments the urea concentrations of the two blood samples differed by less than 10 per cent. In all but three of the experiments upon Necturus and in half of those upon frogs ureteral urine was collected at the end of the experiment from the kidney which supplied tubule fluid; in the others, urine was collected from the bladder and represents the product of both kidneys. In 17 experiments urine was collected both at the beginning and end of the experiment; the latter specimen usually contained the higher urea concentration.

All fluids were analyzed by the ultramicro-modification of the sodium hypobromite method described at the end of this paper. Twelve specimens of fluid from the proximal tubule of Necturus and all specimens of blood plasma and urine were analyzed in duplicate. Urea solutions of known concentration were analyzed with each experiment. In occasional experiments upon Necturus it was necessary to deproteinize tubule fluid and urine (3).

[1] The expenses of this work were defrayed in large part from a grant by the Commonwealth Fund. A preliminary report of these experiments was made before the American Physiological Society in April 1934 (This Journal **109**: 87, 1934).

In interpreting the results of these analyses two sources of error are recognized: the dilution of blood plasma required to deproteinize it diminished their accuracy in 17 experiments upon frogs in which blood plasma contained less than 5 mgm. per 100 cc. urea nitrogen. This error was excluded from the experiments with Necturus by placing the animals in a bath containing 2.0 or 0.2 per cent urea for 1 or 16 hours preceding anesthetization and so increasing the plasma urea concentration. Another and more serious error lies in the fact that in the hypobromite method ammonia is determined as urea. The "urea" concentration ratios are therefore really ratios between the urea + NH_3-N of tubule fluid and urine, and the urea-N of blood plasma. About one-fourth of the urinary "urea" is ammonia (table 4) which must make its appearance at some point within the tubule lumen. The increase in plasma and urine concentrations imposed by the administration of urea minimized this error in the experiments with Necturus.

The average duration of the tubule fluid collection in Necturus was 28 minutes, the average amount of fluid collected 0.64 c.mm. Corresponding figures in frogs were 45 minutes and 0.28 c.mm. The rates of flow within distal tubules of the same kidney and at the same time have been observed to differ markedly; we believe this explains the fact that, in frogs, the rate of collection from the distal tubule was not markedly lower than that from the proximal tubule. The tubule was obstructed distal to the site of collection (2) in 12 of the 41 experiments upon proximal tubule, in 25 of 29 experiments on distal tubule and in 7 of the 11 instances where negative pressures of a few millimeters were used in the collection of fluid. Thirty-one tubule fluids from Necturus and 7 from frog were tested for protein (3); 12 of the former were negative and 3 contained barely perceptible traces; 6 of the latter were negative. In 27 instances the tubule fluid collection was sufficiently large to permit analyses for inorganic phosphate or reducing substances or both in addition to the analyses for urea.

Results. *Necturi.* Table 1 contains the results of 36 experiments arranged according to the portion of the renal unit from which fluid was collected. They show that the urea concentrations of plasma, glomerular fluid and fluid collected from the first quarter of the proximal tubule are essentially the same (nos. 1–6); the concentration of fluid taken from the distal quarter of the proximal tubule is definitely higher than that of plasma (nos. 11–28, av. concentration ratio, 1.28;[2] maximum 1.56); that of fluid from the third quarter of the distal tubule is higher still (nos. 29–36, av. concentration ratio 1.62; maximum 2.61): while the average urea concentration ratio of ureteral urine/plasma is 2.2 (maximum, 5.4). These

[2] The variations which occur are not proportional to the rate at which fluid flowed along the tubule, nor is there any correlation between the Tubule Fluid/Plasma and Urine/Plasma concentration ratios.

TABLE 1

Necturi. Urea concentrations of blood plasma and fluid (T.F.) collected from glomeruli and different levels of the tubules of normal and phlorhizinized Necturi together with urea, glucose and inorganic phosphate concentration ratios

NUMBER	COLLECTION		UREA N IN		CONCENTRATION RATIOS					
	Site	Rate	Pl.	T.F.	Urea		Glucose (phlorhizin)		Inorganic P_2O_5	
					T.F./Pl.	U/Pl.	T.F./Pl.	U/Pl.	T.F./Pl.	U/Pl.
		c.mm./hr.	*mgm./100 cc.*	*mgm./100 cc.*						
1	Glom.	0.96	35.5	34.7	0.98	2.42				
2	Glom.	2.40	9.1	9.4	1.03	2.37				
				Proximal tubule						
3	1/12	1.47	25.0	25.6	1.02	1.64				
4	1/8	1.15	25.0	24.7	0.99	1.64				
5	4.90/20.4	3.42	46.3	54.4	1.18					
6	1/4	1.44	90.9	91.3	1.00	1.51				
7*	1/2	1.84	15.0	16.9	1.13	1.88	1.19	2.18		
8	8.4/15.0	1.07	15.6	17.5	1.13	1.90				
9	11.5/17.4	1.77	15.3	19.7	1.29	3.31				
10*	8.2/11.5	1.01	34.7	38.1	1.09	1.51	1.03	1.91		
11	16.2/19.7	1.00	57.8	76.3	1.32	1.74				
12	12.5/15.1	2.64	14.5	18.7	1.29	2.17			1.19	1.75
13*	7/8	1.19	13.8	17.1	1.24	2.32	1.09	1.88		
14	7/8	0.78	14.9	16.9	1.13	3.31				
15*	9.2/11.6	1.94	20.3	28.1	1.38	2.46	1.44	2.35	1.68	3.68
16	10.8/12.4	2.58	10.7	12.9	1.21	2.20			1.72	1.78
17*	11.4/12.8	1.62	20.0	22.5	1.13	1.47	1.24	1.52		
18*	End	0.88	12.8	16.9	1.31	2.29	1.12	2.10	1.30	2.33
19	11.7/13.1	1.84	20.6	23.1	1.12	1.44			0.96	1.70
20	End	0.91	6.6	10.3	1.56	2.81			1.71	1.84
21*	11.6/12.4	1.14	15.3	23.8	1.55	3.40	1.50	2.93	1.94	3.64
22*	15.7/15.9	2.28	20.6	25.2	1.24	2.19	1.02	1.80		
23**	End		5.0	6.2	1.24					
24	End	0.83	57.8	69.4	1.20	1.74				
25	End	1.92	90.9	116.9	1.29	1.51				
26	End	0.98	19.2	20.0	1.04	2.31			1.09	2.60
27*	End	1.92	19.5	25.8	1.32	1.40	1.05	1.20		
28*	End	1.01	13.1	17.2	1.32		1.40	2.61		
Av.....		1.56			1.26	2.13	1.21	2.15	1.45	2.42

* One hundred twenty-five to 260 mgm. phlorhizin per kilo administered parenterally 6 to 20 hours before experiment.

** No urea administered before experiment.

TABLE 1—*Concluded*

NUMBER	COLLECTION		UREA N IN		CONCENTRATION RATIOS					
	Site	Rate	Pl.	T.F.	Urea		Glucose (phlorhizin)		Inorganic P_2O_5	
					T.F./Pl.	U/Pl.	T.F./Pl.	U/Pl.	T.F./Pl.	U/Pl.
					Distal tubule					
		c.mm./hr.	*mgm./100 cc.*	*mgm./100 cc.*						
29	1/2	0.90	26.9	57.3	2.13	1.95			2.60	3.00
30	1/2	1.26	20.9	23.1	1.10	1.31†			1.14	1.72
31	1/2	0.28	61.4	102.3	1.68	1.94				
32	3.6/6.4	0.63	66.8	77.5	1.16	1.57				
33	3.6/5.2	1.05	17.5	26.3	1.50	1.70			1.62	2.55
34	2/3	0.99	7.9	20.6	2.61	5.43†				
35	2/3	1.24	57.9	77.7	1.34	1.37				
36	5.8/8.0	1.38	12.0	16.9	1.40	2.36†				
Av.....		0.97			1.62	2.20			1.79	2.42

† Ureteral urine not collected from same kidney as tubule fluid.

figures prove that while the concentrating processes are active throughout both segments of the tubule, the greater fraction of the concentration is accomplished in the distal segment.

In 10 of the experiments the animals had been poisoned with phlorhizin and the glucose concentrations of plasma and tubule fluid were also determined. The results show 1, that the concentrating power of the tubule for urea is not influenced by phlorhizin, and 2, that concentration ratios of urea and of glucose are of the same order and in the same range.

Included in the table also are the results of determinations of inorganic phosphate.

Another series of experiments was undertaken in order to learn whether urea can enter or leave the lumen of the proximal tubule through its wall. In group A the lumina were perfused with Ringer's solution containing urea in concentration higher than that of plasma; in group B the Ringer's perfusion fluid contained no urea. The results are given in table 2. They show that while urea is able to pass through the epithelium of the proximal tubule in either direction, equality of concentration in tubule fluid and plasma is more nearly approached when the direction of the gradient is blood → lumen than when it is lumen → blood. The passage of urea from lumen to blood is evidence of the truth of Rehberg's (4) theory of back-diffusion and points to the existence of definite and varying limitations to the concentration of urea which proximal tubules can produce by reabsorption of water.

In 6 experiments of this series the movement of inorganic phosphate was similarly measured at the same time. In 3 of the 4 experiments of group A the percentage of urea which passed out of the tubule was greater than that of phosphate. This reabsorption of urea, rather than secretion of phosphate, is the probable explanation for the fact that the phosphate concentration ratios in table 1 are higher than those of urea particularly since in both of the experiments in which the entrance of the two substances into the tubule was measured the urea concentration attained in the tubule fluid approached more nearly to that of the plasma than did the phosphate concentration.

Frogs (*R. pipiens*). The results of 39 experiments upon frogs are grouped in table 3. The urea concentration of fluid in the proximal tubule rises

TABLE 2

Perfusion of single tubules of Necturus. Passage of urea (and phosphate) through the wall of the proximal tubule

	UREA N IN			INORGANIC P_2O_5 IN		
	Plasma	Perfusion fluid	Perfusate	Plasma	Perfusion fluid	Perfusate
	mgm./ 100 cc.	*mgm./ 100 cc.*	*mgm./ 100 cc.*	*mgm./ 100 cc.*	*mgm./ 100 cc.*	*mgm./ 100 cc.*
A 1	4.8	10.1	9.0	3.6	10.4	6.4
2	3.8	11.3	6.0	4.1	9.9	8.6
3	4.5	20.5	16.5	3.7	10.7	11.6
4	3.7	19.5	9.0	4.1	10.1	5.7
B 1	13.1	0	14.4			
2	25.9	0	17.5	5.1	0	2.4
3	28.2	0	16.0			
4	35.4	0	30.0	4.9	0	3.4
5	36.0	0	40.0			
6	37.1	0	18.0			

above blood plasma, reaching an average concentration ratio of 1.45 in the first half (nos. 4–10) and 2.04 in the last quarter (nos. 11–18).

Eleven specimens of fluid collected from the first half of the distal tubule (nos. 19–29) have an average concentration ratio of 3.44; and 10 fluids collected from the last quarter of this segment (nos. 30–39) show the higher average ratio of 5.08. Within each group there are marked variations independent of the rate of collection but associated with the degree to which the kidney is concentrating urea and this, in turn, is directly related to the plasma urea concentration (see below). The average concentration attained in ureteral urine was 7.84 times that of plasma. It will be noted that here as in Necturus, the degree of urea concentration attained in fluid collected from the distal end of a distal tubule is almost invariably less than

TABLE 3

Frogs. Urea concentrations of blood plasma and fluid (T.F.) collected from glomeruli and different levels of the tubules of normal and phlorhizinized frogs together with urea, glucose and inorganic phosphate concentration ratios

NUMBER	COLLECTION		UREA N IN		CONCENTRATION RATIOS					
	Site	Rate	Pl.	T.F.	Urea		Glucose (phlorhizin)		Inorganic P_2O_5	
					T.F./Pl.	U/Pl.	T.F./Pl.	U/Pl.	T.F./Pl.	U/Pl.
		c.mm./hr.	*mgm./100 cc.*	*mgm./100 cc.*						
1	Glom.	0.70	5.8	6.1	1.06	3.57†				
2	Glom.	0.72	2.8	3.0	1.06	10.80†				
3	Glom.	1.00	7.4	8.0	1.08	6.37				
				Proximal tubule						
4	1/8	0.44	3.8	2.6	0.70	7.00				
5	1/3	0.33	7.4	8.5	1.15	18.80				
6	1/2	0.42	3.9	7.0	1.76	8.80				
7	1/2	0.44	3.1	6.9	2.20	18.60				
8	1/2	0.25	3.8	5.2	1.38	5.88†				
9*	1/2	0.35	6.5	10.1	1.55	5.83†	1.21	2.44		
10*	1/2	0.27	20.7	29.2	1.41	3.93†	1.00	2.87		
Av.					1.45		1.11			
11*	3/4	0.39	39.9	53.3	1.34	2.23†	1.09	1.77		
12	End	0.25	17.1	26.9	1.57	2.98				
13	End	0.22	3.6	3.8	1.06	1.22				
14	End	0.39	13.6	17.7	1.30	3.36				
15	End	1.36	8.8	11.5	1.32	3.65†				
16	End	0.60	2.2	4.2	1.91	10.75†				
17	End	0.12	3.5	14.5	4.33	19.52†				
18	End	0.39	4.0	14.0	3.50	7.45†				
Av.					2.04					
				Distal tubule						
19	1/8	0.40	5.8	17.0	2.93	7.86				
20	1/8	0.38	6.1	7.5	1.23	3.31				
21	1/6	0.20	3.5	12.7	3.68	19.52†				
22	1/4	0.43	28.1	35.7	1.28	1.92				
23	1/4	0.18	10.8	21.4	1.98	1.97†				
24	1/3	0.13	2.7	15.6	5.78	16.78†				
25*	1/2	0.36	19.8	39.8	2.01	3.69	1.15	1.93		

* Three hundred eight to 400 mgm. phlorhizin per kilo injected in anterior lymph sac 2 hours before experiment.

† Ureteral urine from same kidney as tubule fluid.

TABLE 3—*Concluded*

NUMBER	COLLECTION		UREA N IN		CONCENTRATION RATIOS					
	Site	Rate	Pl.	T.F.	Urea		Glucose (phlorhizin)		Inorganic P_2O_5	
					T.F./Pl.	U/Pl.	T.F./Pl.	U/Pl.	T.F./Pl.	U/Pl.
					Distal tubule—*Concluded*					
		c.mm./hr.	*mgm./100 cc.*	*mgm./100 cc.*						
26	1/2	0.15	9.6	14.1	1.47	3.17†				
27	1/2	0.32	7.8	41.0	5.26				1.72	
28	1/2	0.18	6.9	17.4	2.52	11.75				
29	1/2	0.98	3.6	34.8	9.67	18.75			2.00	3.60
Av.					3.44					
30		0.33	8.1	26.9	3.32	6.37				
31	3/4	0.24	3.4	10.8	3.20	4.76†				
32	3/4	1.50	1.5	22.2	14.80	17.53			1.32	1.14
33	3/4	0.54	3.9	9.1	2.31	3.67†				
34	3/4	0.36	6.1	7.3	1.20	3.57†				
35	7/8	0.12	10.9	21.8	2.00	2.43†			1.29	1.50
36	End	0.27	1.5	22.8	15.20	15.33†				
37	End	0.35	13.3	22.5	1.70	2.64†			1.00	1.48
38	End	0.24	6.6	20.9	3.19	4.43				
39	Coll. duct	0.88	3.8	14.4	3.83					
Av.					5.08	7.84		2.25	1.47	1.93

that demonstrated in the ureteral urine. We are inclined to ascribe this to greater rapidity of flow of fluid in the tubule chosen for puncture than in the majority of tubules in the kidney.

In 4 experiments in which phlorhizinized frogs were used glucose was determined and the concentration ratios are recorded. In 5 experiments inorganic phosphate was determined. In both cases the concentration ratios are notably lower than those of urea.

DISCUSSION. The results demonstrate beyond doubt that in both species of Amphibia studied the concentration of urea in the tubule fluid increases progressively as the fluid flows through the tubule and that the greater fraction of the final concentration attained is effected in the distal convoluted segment.

In attempting to show to what extent the results are explainable by one or other of the accepted conceptions of tubule function the two species will be considered separately.

Necturus. The final degree of urea concentration attained is relatively low; the average urine/plasma concentration ratio is 2.20; the maximum observed was 5.4. The average is almost precisely the same as the average

glucose U/P concentration ratio of the 10 phlorhizinized animals. The range of the latter ratios is not as wide as those of urea: but in the series represented by the figures published in another paper (5) one glucose U/P ratio of 5.7 was encountered, and the average of 18 was 2.74. In that paper reasons believed to be conclusive are cited for the conviction that the concentration of glucose in phlorhizinized Necturi is due to water reabsorption solely. If this is accepted it is obvious that enough water is reabsorbed from the glomerular filtrate to account for the concentration of urea which the tubules of Necturus accomplish. The only further assumption required by this explanation is that urea itself is not reabsorbed to a greater degree than glucose.

Objections to this explanation can be based on two grounds: 1. If the simultaneous urea and glucose ratios of proximal tubule fluid are compared, it is found that they are essentially alike in 6 instances; significantly different in 4. None of the differences, however, are due to high urea ratios but rather to the exceptionally low values of the glucose ratio. Hence they are better explained by incomplete phlorhizin action than by secretion of urea.

2. When the lumina of proximal tubules were perfused with artificial solutions, the results (table 2) were such as to indicate that although urea does not enter the proximal tubule in concentrations significantly greater than those in blood it does find readier entrance through the epithelium than escape from the lumen into the blood. Höber (6) made a similar observation in his perfusions of frogs' kidneys and conceives that the wall of the proximal tubule acts toward urea as a valve, open only in one direction. However interesting the phenomenon, it will be remarked that for its exhibition a degree of difference between the urea concentrations of blood and proximal tubule fluid is required which is only encountered in artificially established, abnormal experimental conditions.

Neither in these experiments nor in any others of which we are aware do we find reason for assigning the concentrations of urea in Necturus urine to secretion by the tubule: it is much more credibly attributable to reabsorption of water. In harmony with this statement are the following results of efforts to find direct evidence of secretion of fluid by the proximal tubule of Necturus and to measure directly absorption of fluid from it:

When the circulation in a single glomerulus had been arrested by filling the capsular space with metallic mercury no fluid could be collected from any portion of the proximal tubule, other than the minute amount remaining in the lumen from the period before cessation of glomerular function. This statement is based upon 11 experiments on 7 Necturi in which the periods of attempted tubule fluid collection varied from 5 to 75 minutes and in which intravenous injections were made of 0.9 per cent NaCl and 10 per cent urea solutions.

In 5 instances (4 Necturi, 1 frog) in which the lumen of a single proximal tubule was perfused, we succeeded in measuring with accuracy the amounts of fluid introduced and collected. The difference showed that 26 to 57, average, 40 per cent of the fluid introduced was absorbed.

Frogs. The degree of urea concentration in the frog is so much higher than that encountered in Necturus or than that of other substances in frogs that it is difficult to believe that it results wholly from the reabsorption of water. It would require, on the average, reabsorption by the proximal tubule of 51 from each 100 cc. of glomerular fluid and by the distal tubule of 36 from the remaining 49 cc. Certain other aspects of the results will be pointed out which agree with the belief that the frog's tubule has the capacity of secreting urea into the lumen. On the other hand, it should be kept in mind that high concentration ratios alone do not prove secretion; that calculations of glomerular filtration in amphibia from clearances of xylose or glucose in phlorhizing poisoning may be fallacious; and that we have little reliable quantitative information concerning the movement of water into and out of the tubule through its wall. While the evidence which these experiments afford seems to us to indicate some secretion of urea it can scarcely be called decisive.

1. The product of the rate of collection of tubule fluid multiplied by the T.F./P urea ratio gives a figure which represents the *minimal* rate at which filtration must have occurred in the glomerulus from which the tubule originated if all of the urea found in the tubule fluid was contained in the glomerular filtrate. This value, calculated for experiments 15, 16, 18, 20, 27, 30 and 33 is between 1.1 and 1.7 cu.mm. per hour; for experiment 36 it is 4.1.[3] The highest rates of glomerular fluid collection which have been observed in this laboratory are 3.5 and 4.0 cu.mm. per hour; average rates are less than 1.0 cu.mm. per hour. It is somewhat improbable that such high rates of glomerular filtration as those calculated above even after correction for the NH_3 error (see below) were actually present in so many experiments of one group; hence we are inclined to think the figures cited indicate secretion.

2. Another item which may be taken as evidence of secretion of urea in these experiments is to be found in the inverse relationship which is apparent between the plasma urea levels and the U/P urea concentration ratios (fig. 1). Marshall and Crane (7) were the first to point to this relationship in connection with the excretion of urea and phenol red in frogs. It is characteristic also of the excretion of phenol red (7, 8) and of certain organic iodine compounds (9) in dogs. The proof is satisfactory, particularly in

[3] For experiments 29 and 32 the figures are 9.5 and 22.2 cu. mm. per hour, but in those experiments the tubule was not blocked distal to the site of collection and there is therefore no certainty that all of the fluid collected was derived from one tubule only.

the case of mammals, that a considerable fraction of the excreted phenol red and iodine compounds is secreted by the tubule; hence the relationship has received acceptance as a criterion of secretion. The discovery that the relationship can be identified in the data of our frog experiments and not in those from Necturi provides another argument from our experiments in favor of urea secretion in frogs.

3. Marshall (10) has published simultaneous U/P concentration ratios of urea and glucose in phlorhizinized frogs and of urea and xylose in normal

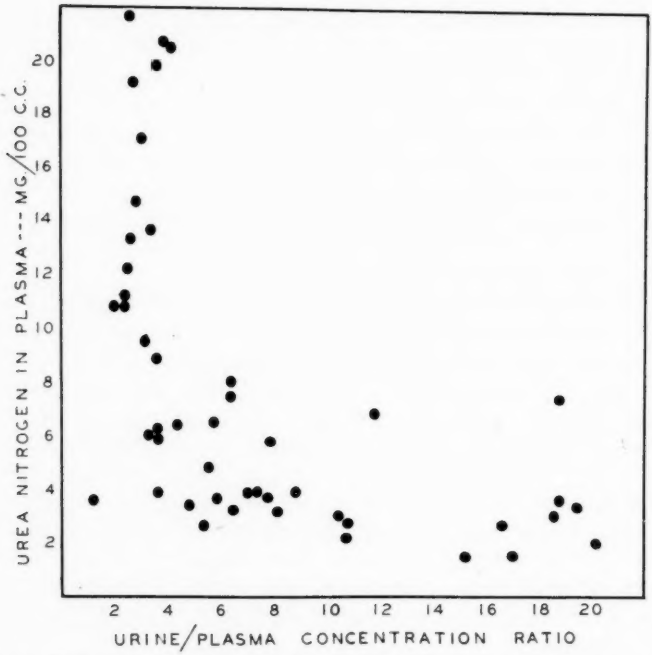

Fig. 1. Illustrating the inverse relationship between the Urine/Plasma concentration ratio of urea and the Urea-N concentration in blood plasma: 47 frogs.

frogs and from these concluded that, in *R. catesbiana*, from 54 to 95 per cent of the excreted urea was secreted. He did not take account of the probable existence of passive reabsorption of glucose or of xylose (5) and for this reason we have doubted the validity of his figures as *proof* of secretion.

Our experiments have yielded data comparable with his. For the phlorhizinized frogs (table 3) simultaneous T.F./P and U/P concentration ratios of glucose and urea are given. If the difference between these is made the basis of calculations, the percentage of excreted urea which was

secreted was from 19 to 58, average 33. Data of additional experiments are gathered in table 4 in which by the use of permutit the NH_3 fraction of the urea + NH_3-N was determined. They give an average of 39 per cent as the fraction of urea which was secreted. This figure is excessive to the extent to which glucose may be passively reabsorbed from the tubule and is therefore cited with the reservation mentioned in referring to Marshall's experiments.

TABLE 4

Secretion of urea calculated from comparison of glucose and urea concentration ratios in phlorhizinized frogs

U/P CONCENTRATION RATIOS			PERCENTAGE OF UREA + NH_3 SECRETED	PERCENTAGE OF UREA SECRETED
Glucose	Urea + NH_3	Urea		
1.19	2.58	2.26	54	47
1.61	2.40	1.83	33	12
1.89	3.82	2.63	50	28
2.14	10.40	7.97	79	73
2.17	7.80	4.02	72	46
2.53	4.10	3.51	38	28
Av....1.92	5.18	3.70	54.3	39.0

SUMMARY

Fluid has been collected from single proximal and distal tubules of Necturus and frog, and its urea concentration compared with that of blood plasma. Sufficient water is reabsorbed by the tubules of Necturus to explain the twofold concentration of urea which its kidney produces. The greater part of this concentration is effected by the distal tubule. The proximal tubule is permeable to urea both in the direction blood-to-lumen and lumen-to-blood.

The frog kidney concentrates urea to an extent which varies enormously with the plasma urea concentration. The concentration proceeds progressively throughout all segments of the tubule but the greater portion occurs in the distal tubule. It is not believed that water is reabsorbed in sufficient amount wholly to explain the concentrations which have been observed. It seems probable that, on the average, something less than 40 per cent of the urinary urea is eliminated by secretion. From the evidence presented it is impossible to conclude whether this urea is secreted by the proximal or distal segments or by both.

AN IMPROVED TECHNIQUE FOR THE ULTRAMICRO-DETERMINATION OF UREA-N. The ultramicro hypobromite method originally designed for the determination of urea-N in fractions of a cubic millimeter of glomerular

urine (1) has been improved by the incorporation of details of the capillary tube technique described by Richards, Bordley and Walker (3). The modified method is more accurate and is applicable to protein-containing solutions. It has been used in all of the determinations reported in the foregoing paper and is exceedingly satisfactory if scrupulous attention is paid to all minutiae of procedure.

Procedure. Sodium hypobromite is prepared according to Stehle (11), 2 cc. of each solution being placed in a 50 cc. Erlenmeyer flask, the mixture gently revolved for 1 minute and placed aside for 30 minutes before use. If the solution to be analyzed contains protein it is deproteinized by tungstic acid (3) or by ultrafiltration through a washed cellophane membrane. Sufficient ultrafiltrate can be obtained from 0.01 cc. of blood plasma.

A scrupulously clean glass capillary tube about 15 cm. in length, with uniform inner diameter of 0.35 mm., is connected with the water manipulator (3) and its open end brought into the optical field of a Greenough binocular microscope (magnification 12×); in the right ocular is a disc micrometer with a 1 cm. scale ruled in 0.1 mm. divisions. With a capillary pipette (3), a column of approximately 60 scale divisions (6 mm.) of distilled water is introduced into the capillary and drawn in about 10 scale divisions from its end. A column of the urea-containing solution, precisely 30 scale divisions (0.3 cu.mm.) long, is then introduced into the capillary from a second pipette, drawn in a short distance, and the end of the capillary sealed with plasticine. While the air column separating the two fluid columns is thus immobilized, its length is accurately measured with a Zeiss filar micrometer temporarily substituted for the right ocular. Two successive readings must agree within one micrometer scale division (5μ). The right ocular is then replaced, the plasticine seal removed by cutting the end of the capillary tube upon the edge of a carborundum block, the column of urea solution forced back to the end of the capillary with the water manipulator, and one volume (30 scale divisions) of sodium hypobromite added to it from a pipette. This pipette has a blunt tip and is freshly filled before use by dipping it into the hypobromite solution. If gas bubbles appear immediately upon the addition of the reagent, the tube should be discarded and fresh reagent prepared. The combined column is withdrawn from the end of the capillary which is again sealed with plasticine. The capillary tube is carefully removed from the water manipulator by cutting it about 6 cm. from its end, revolved for a few seconds between thumb and forefinger, and placed aside in a nearly vertical position upon a plasticine mount. Two hours later the capillary is again revolved for a few seconds, placed horizontally upon the microscope stage, and the length of the air column accurately measured with the filar micrometer. In blank determinations with distilled water this column was found to have increased by an average of 5 scale divisions: for each milli-

gram of urea nitrogen per 100 cc. the increase averages 4 additional scale divisions (20μ).[4] A solution containing 5 mgm. per 100 cc. urea nitrogen should therefore produce an increase of 25 scale divisions in the air column, one of double that concentration, 45 scale divisions. After familiarity with the technique has been gained 16 capillaries can be prepared within one hour and 16 analyses completed within three hours.

Solutions which contain more than 25 mgm. urea-N per 100 cc. should be diluted. If the fluid available measures less than 0.3 cu.mm. it can be

TABLE 5

Comparison of urea determinations by ultramicro- and macro-urease-methods

FLUID	UREA NITROGEN		DIFFERENCE
	Ultramicro-	Macro-urease	
	mgm./100 cc.	*mgm./100 cc.*	*per cent*
Necturus: plasma	13.55	13.85	−2.2
	13.75	15.14	−9.0
	16.25	15.81	2.8
	16.88	16.69	−1.1
	22.13	22.70	−2.5
	25.63	25.50	0.5
Frog: plasma	17.37	16.49	5.3
	20.88	20.09	3.8
	25.58	24.90	2.7
Frog: bladder urine	102.90	106.70	−3.0
	110.40	107.10	3.1
	107.50	111.30	−3.4
	116.80	108.80	7.4
	119.70	113.10	5.8
	130.20	136.40	−4.6
Mean			+0.4
Av. deviation from mean			±3.8

diluted to this volume in the capillary tube, but the N concentration must not be reduced below 2 mgm. per 100 cc.

Tests of method. One hundred thirty-four determinations were made in duplicate upon solutions containing between 2.5 and 25.9 mgm. urea-N per 100 cc. The concentrations of two-thirds of the solutions were unknown to the analyst. The mean error of the series was −0.81 per cent, the standard deviation 4.32. The error exceeded 5 per cent thirty-three times, never

[4] If the procedure released 100 per cent of the nitrogen theoretically present, the increase in length should be 24μ.

exceeded 10 per cent and was no greater with solutions containing less than 10 mgm. urea-N per 100 cc. than with those containing more than this.

Fourteen specimens of blood plasma and bladder urine were analyzed in duplicate by the ultramicro and by the macro-urease-titration method of Van Slyke and Cullen (12) with results which appear in table 5.[5] The difference between the results of the two methods did not exceed 9 per cent and averaged 3.8 per cent. Equally satisfactory results were obtained when ultramicro- and macro-methods were compared by analyses of sterile horse serum. Urea, added in known amount to dialyzed horse serum, was recovered by the ultramicro-method with an average error of 2.3 per cent.

Glomerular fluid and blood plasma from 2 Necturi and 3 frogs were analyzed by the improved method. The results, appearing in tables 1 and 3, confirm the earlier work of Walker and Elsom (1).

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[5] We are indebted to Dr. B. B. Westfall and Miss Ethel Shiels for the macro determinations.

THE RÔLE OF THE TUBULE IN THE EXCRETION OF INORGANIC PHOSPHATES BY THE AMPHIBIAN KIDNEY[1]

ARTHUR M. WALKER AND CHARLES L. HUDSON

From the Laboratory of Pharmacology, University of Pennsylvania, Philadelphia

Received for publication August 25, 1936

Inorganic phosphate is usually concentrated by the kidneys of frog and Necturus. Since this concentration is not effected by the glomerulus (1) it must be effected by the tubule. The experiments of this paper were designed to disclose what portion of the tubule is responsible for this concentration and, if that were possible, to distinguish between the two mechanisms which could produce it, secretion of phosphate by the tubule cells or reabsorption of phosphate-free fluid from the tubule lumen. They have led to the conclusion that both proximal and distal convoluted tubules play a part in the concentration; they indicate that sufficient fluid is reabsorbed to account for the values observed.

METHODS. Blood specimens were collected at the beginning and end of each experiment by venepuncture (Necturus) or cardiac puncture (frog). Tubule fluid was collected by the technique which has been described (2) and its phosphate concentration compared with the average of the two blood samples. Urine was collected from the bladder at the beginning of the experiment or by cannulation or incision of the ureter at the end of the experiment, and its phosphate concentration compared with that of the corresponding blood sample. All specimens were analyzed by the ultramicro-modification of the Bell-Doisy method (1), analyses upon tubule fluid being usually single, upon other fluids duplicate. Approximately half the specimens of tubule fluid and urine from Necturi had to be deproteinized by the addition of one volume of 3.5 per cent trichloroacetic acid.

The average duration of 37 collections of tubule fluid from Necturi was 24 minutes, the average amount of fluid collected 0.46 cu.mm.; corresponding figures in the 15 experiments upon frogs were 37 minutes and 0.21 cu.mm. Sufficient fluid was collected in 14 experiments upon Necturi and 7 upon frogs to permit additional analyses for reducing substances or urea

[1] The expenses of this work were defrayed in large part from a grant by the Commonwealth Fund. A preliminary report of these experiments was made before the American Physiological Society in April 1934 (This Journal **109**: 87, 1934).

TABLE 1

Phosphate concentrations of blood plasma and fluid (T.F.) collected from glomeruli and different levels of the tubules of Necturi and frogs

NUMBER	SITE OF COLLECTION	PHOSPHATE IN		CONCENTRATION RATIOS	
		Plasma	T.F.	T.F./Pl.	U./Pl.
		Necturi			
		mgm. per cent	*mgm. per cent*		
1	Glom.	4.4	4.6	1.05	1.63
2	Glom.	2.4	2.5	1.04	4.28
3	Glom.	2.7	2.9	1.07	1.96
Av.				1.05	
		Tubule neck			
4	End	3.2	3.1	0.97	4.08
5	End	3.9	4.2	1.08	1.72
Av.				1.03	
		Proximal tubule			
6	1/8	2.4	2.5	1.04	1.65
7	1/4	2.4	2.4	1.00	1.65
8	1/4	3.9	4.5	1.15	
9	1/4	3.0	3.8	1.27	2.14
Av.				1.11	
10*	2/3	11.2	13.5	1.21	2.00†
11	13.3/16.9	2.2	2.6	1.18	1.95†
12	12.5/15.1	4.0	4.75	1.19	1.73†
13	12.7/15.4	2.8	3.4	1.21	4.08
14	11.7/13.1	2.7	2.6	0.96	1.70†
15	16.7/17.7	3.0	4.1	1.37	1.96
16	7/8	1.7	2.3	1.35	2.13
17	15.9/——	2.4	3.3	1.37	6.00
18	End	3.6	4.3	1.19	2.00
19	End	3.2	4.0	1.25	1.71
20	End	3.3	4.5	1.36	1.33
21	End	4.4	6.0	1.35	1.63
22	End	3.1	4.2	1.36	2.14
23	End	3.5	3.8	1.09	2.60†
24	End	4.1	7.0	1.71	1.71†
25**	7.0/10.5	3.5	6.5	1.86	3.09†

* Fifty mgm. NaH_2PO_4 injected subcutaneously 2 hours before experiment.

** Fifty-four to 450 mgm. phlorhizin per kilo injected intraperitoneally 4 to 18 hours before experiment.

† Ureteral urine from same kidney as tubule fluid. Other figures in this column represent bladder urine.

TABLE 1—*Concluded*

NUMBER	SITE OF COLLECTION	PHOSPHATE IN		CONCENTRATION RATIOS	
		Plasma	T.F.	T.F. Pl.	U. Pl.
		Proximal tubule—*Continued*			
		mgm. per cent	*mgm. per cent*		
26**	6/8	1.8	2.2	1.22	2.50†
27**	9.2/11.6	3.8	6.4	1.68	2.00
28**	13.6/16.2	3.3	6.3	1.91	6.36†
29**	7/8	1.9	2.25	1.18	3.90†
30**	7/8	2.3	2.4	1.04	1.47
31**	7/8	3.3	4.3	1.30	2.33†
32**	11.6/12.4	3.3	6.4	1.94	3.64†
Av.				1.36	
		Distal tubule			
33	4.4/—	2.5	5.2	2.08	3.36†
34*	1/2	11.2	16.5	1.47	2.00†
35	1/2	4.3	11.2	2.60	3.00†
36	3.6/5.2	2.4	3.8	1.62	2.50†
37*	2/3	11.2	18.4	1.64	2.00†
Av.				1.88	2.55
		Frogs			
		Proximal tubule			
38	1/3	2.4	2.6	1.08	2.83
39	2/3	2.3	3.0	1.30	3.87
40	3/4	3.6	3.8	1.06	1.22
41	End	3.0	3.6	1.20	1.53
42	End	2.0	2.7	1.33	1.90†
Av.				1.20	
		Distal tubule			
43	0.05/6.5	3.1	4.5	1.45	1.65
44	1/4	2.5	2.6	1.04	1.84
45	1/2	5.8	10.0	1.72	
46	1/2	2.5	4.8	1.92	3.60
47	3/4	2.4	3.1	1.29	1.50†
48	End	2.7	3.7	1.37	2.80
49	End	2.8	2.8	1.00	1.50†
50	End	2.25	3.8	1.66	1.88†
51	End	3.9	4.6	1.20	1.10†
52	Coll. duct	3.2	7.8	2.44	1.70†
Av.				1.51	2.06

or both. Seventeen of the 29 fluids which were tested for protein (3) contained none or less than one-fiftieth as much as blood plasma. The tubule was blocked peripherally to the site of fluid collection in 12 of the 15 experiments upon distal tubules and in 11 of the 18 instances in which a few millimeters of negative pressure were used during the collection.

RESULTS. The results of the analyses upon both Necturus and frog appear in table 1.

There is no increase in the phosphate concentration of glomerular fluid (expts. 1–3) as it passes through the tubule neck (expts. 4–5). Nor is the increase distinct in the first quarter of the proximal tubule (expts. 6–9, 38). But as the end of the proximal tubule is reached the increase becomes considerable. Twenty-three fluids collected from the last third of this seg-

TABLE 2

*Concentration ratios of sugar and phosphate in Necturi poisoned with phlorhizin**

PROXIMAL TUBULE FLUID/PLASMA		URETERAL URINE/PLASMA	
Reducing substances	Phosphate	Reducing substances	Phosphate
1.09	1.18	2.10	2.33
1.12	1.04	2.35	3.68
1.12	1.30	2.43	3.90
1.23	1.22	2.61	2.71
1.44	1.63	2.88	2.50
1.50	1.94	2.93	3.64
1.52	1.91	3.09	1.72
2.06	1.86	3.62	3.09
		5.70	6.36
Av.....1.39	1.51	3.08	3.33

* Fifty-four to 450 mgm. per kilo injected intraperitoneally 4 to 18 hours before experiment.

ment in Necturus have an average fluid/plasma concentration ratio of 1.36 and 4 fluids from an analogous site in frogs 1.23 (expts. 10–32, 39–42). Variations amongst the individual results are but slight in two-thirds of the experiments and are unrelated to the rates at which fluid was collected, though these ranged from 0.37 to 2.60 cu.mm. per hour, or the degree to which the kidney was concentrating phosphate.[2]

[2] Eight Necturi (nos. 25–32) had been poisoned with phlorhizin and were therefore not actively reabsorbing glucose. Four of the 5 highest tubule fluid/plasma and 5 of the 7 highest ureteral urine/plasma phosphate concentration ratios occurred in these animals. This could be adduced as an argument favoring phosphoryllation as the mechanism of glucose reabsorption (4) were the relationship more constant and were it not absent from the bladder urine/plasma concentration ratios.

In the distal tubule the concentration is carried further. Five fluids collected from its first two-thirds in Necturus have an average fluid/plasma ratio of 1.88 (nos. 33–37), 10 fluids collected from throughout its length in frog a ratio of 1.51 (nos. 43–52). In both animals the degree of concentration shown by distal tubule fluid lies roughly midway between those of proximal tubule fluid and of urine.

These results provide an answer for the question which was posed. Both proximal and distal convoluted tubules take part in the concentration of phosphate. The distal tubule appears to be somewhat more effective than the proximal since, to produce the average concentration ratios existing at the end of the proximal tubule (1.32) and at the ureter (2.41), the proximal tubules must reabsorb 25 of each 100 cc. of glomerular filtrate, and the

TABLE 3

Perfusion of single tubules of Necturus. Passage of phosphate through the wall of the proximal tubule

	PHOSPHATE IN		
	Plasma	Perfusion fluid	Perfusate
	mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*
A	2.4	0.0	0.4
	2.5	0.0	1.3
	3.5	0.0	2.5
	4.5	0.0	2.9
	4.6	0.0	0.8
	4.9	0.0	3.4
B	3.6	10.4	6.4
	3.7	10.7	11.6
	4.1	10.1	5.7
	4.3	10.0	8.8
	4.6	9.5	8.2

distal tubules 46 of each 100 cc. which reaches them from the proximal tubules. In attempting to determine whether water is reabsorbed in amounts of this order we employed measurements of sugar concentration in animals poisoned with phlorhizin; in exploring the opposite hypothesis, that phosphate is secreted, we employed the perfusion of single tubules.

Phlorhizin experiments. The results of the 9 experiments in table 2 indicate that, on the average, phosphate and reducing substances are concentrated to a very similar degree both in proximal tubule fluid and in urine of Necturi poisoned with phlorhizin. Since the concentration of reducing substances under these circumstances may be taken as a *minimum* measure of water reabsorption (5) it appears that sufficient water is reabsorbed to explain at least 90 per cent of the observed phosphate concen-

trations and the remaining 10 per cent may quite as well be due to the passive reabsorption of reducing substances (5) as to the active secretion of phosphate. Examination of the individual experiments discloses that the agreement is much less perfect than is indicated by the averages. Although this might be interpreted to mean that some kidneys secrete phosphate, others sugar, it is also explicable by such variations in tubule permeability to phosphate as are demonstrated in the perfusion experiments to be described (table 3 B).

The results of experiments with frogs poisoned by phlorhizin are less open to debate. The reducing substance concentration ratios of both proximal tubule fluid (1.40) and ureteral urine (2.99) in phlorhizinized animals (5) are so far above the phosphate concentration ratios of these fluids in normal animals (1.20 and 2.08, table 1) that we must conclude there is more than sufficient water reabsorption to account for the phosphate concentration.

Perfusion experiments. If phosphate were secreted by the proximal tubule of Necturus, it should appear within the lumen of a tubule perfused with phosphate-free solutions. In table 3 A are the results of 6 experiments in which such perfusions were carried out by the technique previously described (2). In each case phosphate was present in fluid collected from the end of the tubule, but in amounts which averaged only one-half of the plasma concentration. It is impossible to say whether this phosphate entered the tubule by diffusion or secretion. Since the concentration never approached that of blood plasma it is plausible to believe that the mechanism was diffusion; and the experiments presented in table 3 B favor this hypothesis for it appears that phosphate can leave tubules perfused with solutions containing a concentration greater than that of blood plasma.

Active reabsorption of phosphate. Although the bladder urine of Necturi and frogs usually contains a higher concentration of phosphate than does blood plasma, a considerable number of Necturi and 2 frogs have been encountered in which the reverse was true. An analogous situation, observed in mammals, has been attributed (6) to an inability on the part of glomeruli to filter phosphate from the plasma. This is not the explanation of the situation in Necturi, for in two experiments in which the bladder urine was nearly free of phosphate, glomerular fluid contained it in the same concentration as did blood plasma. The explanation lies in an active reabsorption of phosphate by the tubule, for in 6 animals which were showing this phenomenon, specimens of proximal tubule fluid contained distinctly lower phosphate concentration than blood plasma.[3] The process

[3] The reabsorption is not necessarily present in all tubules of the same kidney or in the same kidney from time to time, for one animal was seen in which one proximal tubule contained fluid less concentrated than plasma while a second contained

is apparently unrelated to the concentration of phosphate in the blood plasma; in one animal it was not obviated by the intravenous injection of 5 units of Parathormone.

SUMMARY

Fluid was collected from various portions of single renal tubules in Necturus and frog. Its phosphate concentration, compared with that of blood plasma, rises progressively as it passes through proximal and distal tubules towards the ureter. Reasons are advanced for the belief that, in both animals, this concentration is effected by the reabsorption of phosphate-free fluid rather than by the secretion of phosphate. The distal tubule plays a somewhat larger rôle than the proximal tubule in the process.

Perfusion of single proximal tubules in Necturus indicates that phosphate may pass through them both in the direction blood to lumen and lumen to blood.

The proximal tubules of both animals are capable of actively reabsorbing phosphate. It is this reabsorption rather than a failure of glomerular filtration which accounts for the occasional finding of a bladder urine with phosphate concentration below that of blood plasma.

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fluid more concentrated; another in which proximal tubule fluid was more concentrated than blood plasma while ureteral urine, simultaneously formed was less; a third in which the reverse was true; and 3 animals in which bladder urine was less concentrated than blood plasma but urine collected from the ureter an hour later was more concentrated.

THE MECHANISM OF CONVULSIONS IN INSULIN HYPOGLYCEMIA

Interrelationship of Blood Concentration, Cerebrospinal Pressure and Convulsions[1]

DAVID L. DRABKIN and I. S. RAVDIN

From the Department of Physiological Chemistry and the Harrison Department of Surgical Research, Schools of Medicine, University of Pennsylvania, Philadelphia

Received for publication August 26, 1936

In a study of insulin anhydremia Drabkin and Shilkret (1) made the observation of the absence of spastic convulsions in dogs which had been deprived of water and then rendered severely hypoglycemic by the administration of insulin. Interest in this finding was increased by the possible relationship of water balance and the epileptic state (2).

The present study was undertaken in an attempt to elucidate the mechanism of convulsions in insulin hypoglycemia. The original findings of Drabkin and Shilkret (1) were confirmed and greatly extended. Data have been secured which suggest the following sequence of events in hyperinsulinism: hypoglycemia—anhydremia—rise in cerebrospinal pressure—convulsions. Other factors, however, materially complicate this relatively simple picture.

Experimental procedures and methods. A total of 40 experiments were performed upon 31 dogs, which varied in weight from 6 to 26 kgm. In each case the experiment was a study of the response, during a period of approximately four hours, following the intravenous administration of 20 units per kilogram of body weight of insulin (Eli Lilly & Company). The preparation of the animals before the administration of insulin varied considerably and may be summarized under five main groups as follows:

1. Ten experiments were upon 6 relatively hydrated animals. In addition to receiving drinking water *ad libitum*, animals, designated as hydrated, were given by stomach tube 40 to 80 cc. of water per kilogram per day, for five to seven days.

[1] A preliminary report of this work was presented at the Chicago Meeting of the Federation of American Societies for Experimental Biology, and an abstract of the report has appeared in J. Biol. Chem. **87:** III, 1930. We have delayed the final publication of our data in the hope, not realized, that further experiments would reveal a more positive explanation for our findings in that portion of the study which deals with the section and extirpation of elements of the autonomic nervous system.

2. Nine experiments were performed upon 6 relatively dehydrated animals which were deprived of water, but not of food, for a period of five to seven days before the administration of insulin. Animals in this group often showed a mild degree of anhydremia, due to the water restriction (1).

3. Thirteen experiments were carried out upon eleven hydrated animals which previously had undergone complete, bilateral removal of the stellate ganglia. Before stellate ganglionectomy two of the animals had been subjects of experiment as hydrated non-operated animals of group I. A period of ten to fifteen days following operation was allowed for complete wound healing and hydration (as in group I) before the administration of insulin.

Operation for excision of the stellate ganglia. Ether anesthesia was used. An incision, extending for about six inches, was made along the mesial border of the scapula. The muscles were dissected free from the chest wall so as to expose the first, second, third, and occasionally, the fourth ribs. The second rib was freed of its periosteum, and the posterior portion of the rib, about 5 cm. in length, was resected. Using Frazier lighted retractors, the sympathetic chain was located and followed to the stellate ganglion. The connections to the ganglion were divided, and the ganglion excised. Wound closure was by tier suture, without drainage. After suturing, the stellate ganglion of the opposite side was excised by the same technique. Since the operation was extrapleural, positive pressure was not required. A persistent bilateral enophthalmus following excision of the ganglia was not relied upon as a sole index of the completeness of the operation. The results were verified in all cases by subsequent exploration.

4. Five experiments were carried out upon five hydrated animals which previously had undergone sectioning of both splanchnic nerves. Two of the animals in this group had served as subjects of experiment as hydrated animals with stellate ganglia excised of group III. The splanchnic nerves were sectioned in their thoracic portion by an extrapleural approach through an incision parallel with the tenth rib. The high section is necessary to insure severance of the splanchnic innervation to the adrenals. The operative technique, developed in the Department of Surgical Research, has been described (3). Complete recovery of the animals from the operation was permitted; they were then hydrated (as in group I) before the administration of insulin.

5. Three experiments were performed upon three hydrated animals which had been subjected both to bilateral stellate ganglionectomy and bilateral sectioning of the splanchnic nerves. Two of these animals had previously served in experiments in group I and group III.

A record was kept of the symptoms and behavior of the animals during the progress of hyperinsulinism. In most of the experiments where no

anesthesia was used the animals were permitted freedom of movement about the laboratory, except for the relatively brief intervals when drugs were injected or blood for analysis was withdrawn from the saphenous vein. Under the conditions of our experiments (large doses of insulin, etc.), we have observed no correlation between the posture of the animal and the incidence or severity of the resultant anhydremia after insulin (4). The blood was analyzed for total reducing substances by the Shaffer and Hartmann method (5), using the modified reagent described by West, Scharles and Peterson (6). Since we were interested only in relative reducing values no attempt was made to concentrate the protein-free filtrate so as to insure accuracy in extremely hypoglycemic samples. Occasional samples gave no reduction by the technique used; such samples were no doubt practically sugar-free, but not free of saccharoids (7). The hemoglobin content of the blood samples was used as an index of relative blood concentration and was determined by a slightly modified Newcomer technique (8), using a plate recalibrated by one of us spectrophotometrically (9). The results are reported in per cent, 100 per cent being equivalent to 13.8 grams of hemoglobin per 100 cc. of blood.

Of the objective records of the response to insulin the most important from our present standpoint was that of cerebrospinal fluid pressure. Discontinuous records, such as pressures at the inception and termination of a particular experiment (as in table 1), were obtained with the animal under transitory, very light ether anesthesia. Continuous records (as in figs. 1 and 2) were obtained from animals anesthetized with sodium amytal, 50 mgm. per kilogram of body weight. The records were read from a calibrated water manometer in direct connection with the spinal fluid through a needle inserted into the cisterna magna and rigidly fixed in position. The curves which are presented are simplified by averaging to exclude the respiratory excursion, which served as an index of needle patency and position. When the respiratory excursion was very small, as in the later stages of most of the experiments, the Quackenstedt reaction[2] was used to confirm the continuity of cerebrospinal fluid and manometer. The records were obtained with the anesthetized animal in the horizontal position, lying upon its abdomen on a special table.[3]

Minor procedures of some interest were the following: In two experiments, after the development of insulin anhydremia and convulsions, the effect of the intravenous injection of 40 cc. of 25 per cent, buffered sodium arabinate (Smith, Kline and French) was studied. This agent was employed to raise the blood volume without direct influence upon the blood sugar content. In several experiments 0.1 cc. of oil of wormwood per

[2] Pressure over the animal's jugular vein reflected by simultaneous increase in manometer reading.

[3] Designed by Dr. R. M. Lewis.

TABLE 1

The effect of extirpation of the stellate ganglia and of sectioning of the splanchnics upon the occurrence of spastic convulsions after large doses of insulin

The changes in blood sugar, blood concentration and cerebrospinal pressure are shown.

DOG NUMBER	BEFORE INSULIN		HOURS AFTER INSULIN								C.S.P.‡		CONVULSIONS	PROCEDURES BEFORE INSULIN
			1		2		3		4					
	G*	Hb †	G	Hb	G	Hb	G	Hb	G	Hb	Initial	Terminal		
2	98	95	49	99			18	117	0††	126	167	295	Spastic	Animal hydrated
5	118	109	57	130			28	141	22	157	65	0	None	Animal dehydrated
6	118	101	41	110	34	138							Severe spastic	Left stellate ganglion removed; animal hydrated
7	99	86			110	84	80	89					None	Both stellate ganglia removed; vagi sectioned; animal hydrated
7	111	81	88	81	50	77	50	65	43	83		110	None	Same as preceding experiment
8	83	86	66	89	67	79			57	78			None	Both stellate ganglia removed; animal hydrated
9	90	73	72	71	66	70	49	66	47	66			None	Both stellate ganglia removed; animal hydrated. Convulsions produced with wormwood oil
10	87	88			46	102	18	121					Severe spastic	Both splanchnic nerves sectioned; animal hydrated
11	104	96	36	128	16	138							Severe spastic	Both stellate ganglia removed; both splanchnic nerves sectioned; animal hydrated after stellate ganglionectomy, convulsions produced with wormwood oil
12	110	102			112	100	68	115	55	121			None	Both stellate ganglia removed; animal hydrated
13	122	100	102	98	88	103	47	106					None	Both stellate ganglia removed; animal hydrated
13	104	101	48	110	18	126							Severe spastic	Both stellate ganglia removed; both splanchnic nerves sectioned; animal hydrated
14	103	96	40	113	22	115							Severe spastic	Animal hydrated
14	122	100	94	102	83	105	41	109					None	Both stellate ganglia removed; animal hydrated
15	99	102	54	106							135	265	Spastic	Animal hydrated
15	102	94	74	96			58	94				150	None	Both stellate ganglia removed; animal hydrated
15	106	94			22	108					170	290	Severe spastic	Both stellate ganglia removed; both splanchnic nerves sectioned; animal hydrated
16	108	100	38	115	32	106	28	113					Severe spastic, before arabinate administration	Animal hydrated; sodium arabinate injected between third and fourth blood samples

*G : Total reducing substances in blood in milligrams per 100 cc.

† Hb: Hemoglobin in per cent.

‡ C.S.P.: Cysternal pressure in millimeters of water, taken under light ether anesthesia. "Initial" indicates about one-half hour prior to drawing of first blood sample; "terminal" about one-half hour after last blood sample was drawn.

†† Too low a magnitude for determination by technique used

kilogram of body weight was administered to animals which had their stellate ganglia excised and had reacted to insulin as described under experimental results. This drug is believed to produce convulsions by direct cerebral irritation (10) in contrast to the convulsant action of insulin. In four of the experiments in which continuous cerebrospinal pressure records were taken, simultaneous kymographic records of blood pressure were made. In practically all the experiments recovery from hyperinsulinism, after a period of about four hours, was induced by

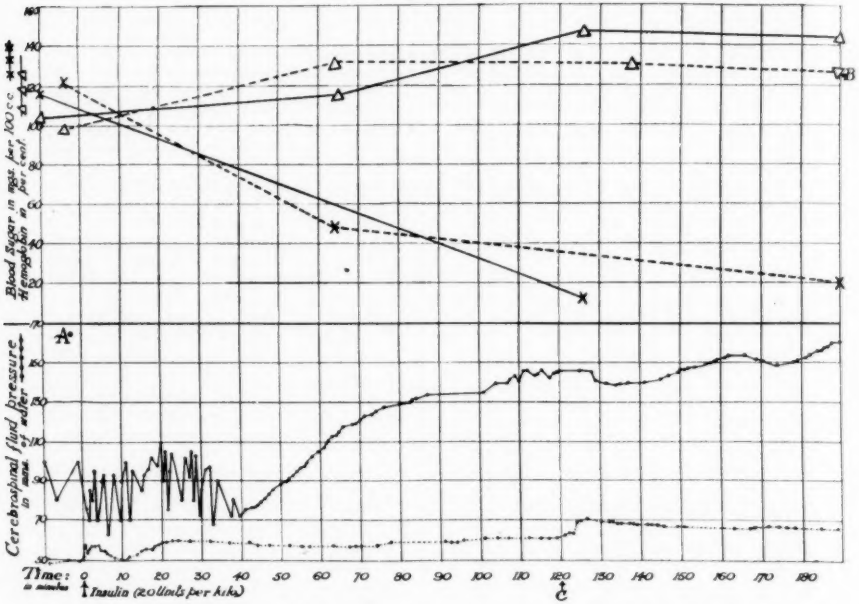

Fig. 1. Changes in blood sugar, blood concentration and cerebrospinal pressure after the administration of insulin. Solid lines: observations on dog 1, hydrated. Broken lines: observations on dog 2, dehydrated. A—initial cerebrospinal pressure of dog 2, when hydrated; B—cerebrospinal pressure, 3 hours after the administration of insulin, of dog 2, when hydrated; C—time of onset of convulsions after insulin administration in dog 2, when hydrated.

intraperitoneal administration of sterile glucose solution (300–500 cc. of 10 per cent of Pfanstiehl C.P. anhydrous d-glucose).

Experimental results. Figures 1 and 2 present data typical of the results in experiments upon hydrated and dehydrated animals from which continuous records of cerebrospinal fluid pressure were obtained during hyperinsulinism. To simplify the figures blood pressure records, also obtained, are omitted. In both hydrated and dehydrated animals after massive doses of insulin severe hypoglycemia and anhydremia developed

A striking difference was observed, however, in the records of cerebrospinal fluid pressure. In hydrated animals (nos. 1 and 3) the cerebrospinal pressure invariably rose markedly after insulin. In dehydrated animals, during hypoglycemia, the pressure rose slightly (no. 2), remained unchanged or fell (no. 4). The blood pressure of hydrated animals was maintained at pre-insulin levels, while in one dehydrated animal it fell markedly during the first hour after insulin.

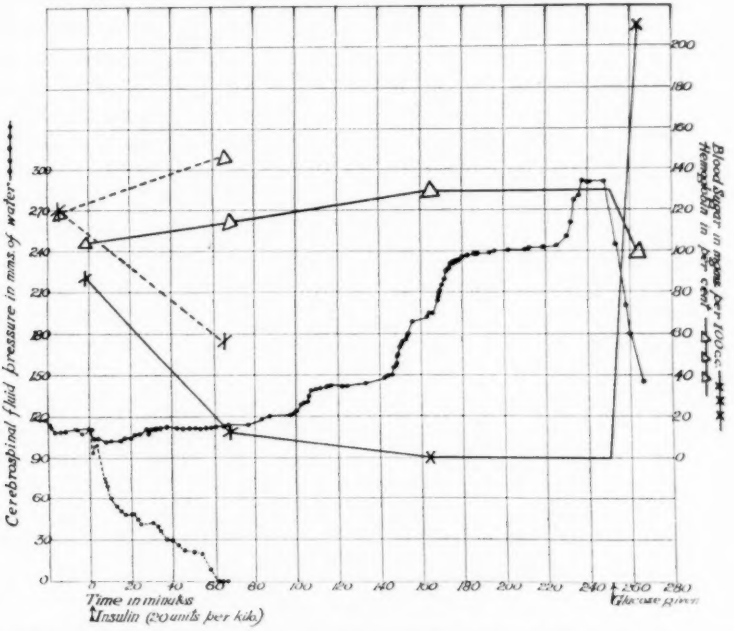

Fig. 2. Changes in blood sugar, blood concentration and cerebrospinal pressure after the administration of insulin. Solid lines: observations on dog 3, hydrated. Broken lines: observations on dog 4, dehydrated.

Table 1 presents a synopsis of representative experiments upon unanesthetized animals. Convulsions were invariably produced in insulinized, hydrated animals (7 expts.) and were absent in dehydrated animals given insulin (6 expts.).[4] By running experiments upon the same animal (example 2, fig. 1 and table 1) when in the dehydrated and hydrated state, an unequivocal correlation appeared between state of hydra-

[4] Dehydrated animals after insulin rapidly pass into an apathetic condition, which leads to coma. The administration of glucose produces recovery in these animals. The absence of convulsions in these animals during hypoglycemia constitutes the main clinical difference between them and hydrated animals given insulin.

tion, level of cerebrospinal fluid pressure and incidence of convulsions. The time of onset of convulsions in hydrated animals (fig. 1) was roughly coincident with the attainment of maximum hypoglycemia, blood concentration and cerebrospinal fluid pressure. However, hypoglycemia *per se* was definitely not responsible for the convulsions. The absence of convulsions in severely hypoglycemic dehydrated animals made this evident, but further proof was furnished by the sodium arabinate experiments (example 16, table 1). After the injection of sodium arabinate into convulsant, hypoglycemic animals, convulsions disappeared during the 30 to 45 minutes that this agent was exerting its action of drawing fluid into the blood stream, thereby decreasing the cerebrospinal pressure, but without change in the hypoglycemia. Anhydremia increased and convulsions reappeared when the sodium arabinate was no longer effective. It should also be pointed out that hypoglycemia was probably not solely responsible for the anhydremia after insulin. In comparable experiments, involving similar large doses of insulin, a marked hypophosphatemia, 55 to 75 per cent decrease in inorganic phosphate and a reduction in CO_2 capacity of the blood, of the order of 20 to 30 per cent, usually occurred in the later stages of the experiment (11).

In thirteen experiments upon eleven animals we have obtained the unexpected result that the excision of both stellate ganglia strikingly modifies the response to large doses of insulin. These animals, though hydrated, had no convulsions after insulin. During an observation period of three to four hours they walked about the laboratory and were playful, exhibiting no sign of reaction to the drug. While hypoglycemia developed, it may be seen (table 1, nos. 8, 9, 12, 13, 14 and 15) that its onset was delayed and that it was definitely less severe in comparison with non-operated, hydrated animals (no. 2 and no. 14, before operation). It is also evident that in these animals there was little or no change in blood concentration and probably no change in cerebrospinal fluid pressure (nos. 7 and 15). Animal 7 had, besides bilateral stellate ganglionectomy, both vagi sectioned, but this additional operation did not further alter his reaction to insulin. It is also of some interest to note that these animals were still susceptible to convulsive response with such agents as oil of wormwood. Excision of but one stellate ganglion (no. 6) was not effective in modifying the usual reaction to insulin.

Hydrated animals, which previously had both splanchnic nerves sectioned (no. 10), developed unusually severe convulsions after insulin. From the objective records of blood sugar and hemoglobin concentration, however, we cannot conclude that such animals were more than normally sensitive towards the drug.

Animals (nos. 11, 13, and 15), which previously had undergone both excision of the stellate ganglia and section of the splanchnic nerves,

reacted after hydration and administration of insulin similarly to non-operated hydrated animals. This was strikingly demonstrated by a comparison of the data upon animals 13 and 15 after stellate ganglionectomy and after the further operation of splanchnic section. Following the latter operation, once again the typical reaction of pronounced hypoglycemia, rise in blood concentration and cerebrospinal fluid pressure, and severe convulsions was obtained.

Discussion. Our experiments have disclosed a sequence of events leading to convulsions in hyperinsulinism. These may be outlined as follows: severe hypoglycemia (plus hypophosphatemia and decrease in CO_2 capacity of the blood) → anhydremia → rise in cerebrospinal fluid pressure to a critical level → (unknown mediating factors) → convulsions. Convulsions do not occur when this cycle is interrupted, although severe hypoglycemia may be present. Such agents as sodium arabinate interfere with the cycle by preventing or relieving the anhydremia. Dehydration presumably interferes with the cycle by preventing the rise of cerebrospinal fluid pressure to a critical level. This sequence of events does not apply to convulsions induced by such drugs as oil of wormwood, which is probably a direct cerebral irritant.

"Unknown mediating factors" has been included in the above cycle to allow for such influences as disclosed in our experiments upon excision and section of parts of the autonomic nervous system. It should be pointed out that these mediating factors may play a rôle at any or all stages of the cycle and are not implied to operate solely between the cerebrospinal pressure and the convulsive response.

Section of the splanchnic nerves, as performed by us, interferes with the innervation of the adrenals thereby removing or reducing their accepted antagonistic effect with reference to insulin activity. This explanation is, we realize, probably incomplete. We have no entirely satisfactory explanation for the effects of stellate ganglionectomy. The delay in onset of hypoglycemia and definite reduction in its severity is the fundamental point in these experiments. This change in response to insulin we believe adequately accounts for the other findings, absence of anhydremia, absence of rise in the cerebrospinal pressure and absence of convulsions. It has been shown in earlier experiments (12) that the production of a sudden, profound hypoglycemia is essential for the development of anhydremia after insulin. Thus, in animals with their stellate ganglia excised, the sequence of events leading to convulsions may be said to have been blocked at its very inception.

The type of blood sugar response obtained after insulin strongly suggests that, following stellate ganglionectomy, animals are rendered relatively resistant to this agent. To explain this resistance to insulin the following hypotheses are offered tentatively: The stellate ganglia, when

intact, exert either a stimulating effect upon the thyroid, or an inhibiting effect upon the hypophysis in regard to carbohydrate metabolism, or both. Excision of both stellate ganglia may remove the adjuvant effect which the thyroid may normally have in carbohydrate metabolism, rendering the animal more resistant to insulin. The hypophysis may be looked upon as normally exerting an effect upon carbohydrate metabolism which is antagonistic to the effect of insulin. This effect of the hypophysis may be mediated through the adrenals, also antagonistic to insulin. Extirpation of the stellate ganglia would permit the hypophysis to overfunction in regard to carbohydrate metabolism, thereby increasing resistance to insulin. The section of the splanchnic nerves, on the other hand, would do away with the effect of stellate ganglionectomy by blocking a mediation of the pituitary action via the adrenals.

Whatever may prove the eventual explanation of the above experiments, it is of interest that, except for an effect upon lactation, Cannon and his co-workers (13) found that total sympathectomy produced no important, physiological changes in animals subjected to the operations. On the other hand, paradoxically, in our experiments relatively profound changes were produced by exclusion of only parts of the autonomic system. The effect may be likened to a running horse in harness. If both reins snap, though unguided, he may continue along the proper course; if but one rein is broken, trouble may ensue unless the hold is completely loosed upon the remaining rein.

SUMMARY

The previous state of "hydration" of an animal had a profound effect upon the incidence of convulsions in insulin hypoglycemia. "Dehydrated" animals did not have convulsions.

The experiments disclosed the following sequence of events leading to convulsions in hyperinsulinism: severe hypoglycemia → anhydremia → rise in cerebrospinal fluid pressure to a critical level → convulsions. Other factors, however, probably also function in the cycle, although they have not been disclosed. The administration of such agents as sodium arabinate, the practice of dehydration and excision of the stellate ganglia interfered with the above cycle, at different points, and thereby prevented a convulsive response in insulin hypoglycemia.

The excision of both stellate ganglia markedly modified the animal's response to insulin. Data are furnished which indicate that this procedure rendered the animals relatively resistant to the drug. The further operation of bilateral splanchnic section once again rendered the animals normally reactive to insulin.

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INFLUENCE OF ADRENALECTOMY ON THE KETOSIS OF FASTING AND ON THE ACTION OF THE ANTERIOR PITUITARY KETOGENIC PRINCIPLE

EATON M. MacKAY AND RICHARD H. BARNES

From The Scripps Metabolic Clinic, La Jolla, California

Received for publication September 8, 1936

Burn and Ling (1, 2) and later Anselmino and Hoffman (3) demonstrated that extracts could be obtained from the anterior lobe of the pituitary which have the property of producing a ketosis in rats or rabbits which are fasting or receiving a high fat diet. This ketogenic factor cannot be identical with the anterior pituitary thyrotropic hormone for Funk (4) found that it is active in thyroidectomized rats and Butts, Cutler and Deuel (5) report an insignificant ketonuria from the thyrotropic hormone. The experiments of Long and Lukens (6) in which a reduction in the urinary ketones occurred after adrenalectomy in depancreatized cats, Evans' observation (7) that removal of the whole adrenals but not of the medulla alone decreases ketogenesis in phloridzinized animals and our study (8) in which it was shown that removal of the adrenals abolishes the ketosis of late pregnancy, all make the presence of the adrenals of importance in relation to the occurrence of all types of ketosis and particularly that due to extracts of the anterior pituitary. Observations have been made upon this point and incidentally on the influence of adrenalectomy upon the ketosis of simple fasting.

EXPERIMENTAL. Urine collections were made under light mineral oil and the ketone bodies determined by Van Slyke's method (9) which was also used for blood determinations (10). The data presented in table 1 are typical of the results obtained. The rats used were all females because of the greater ease with which ketosis is produced in this sex (11). Essentially similar results have been obtained on male rats. The adrenals were removed under ether anesthesia in the usual manner (12). In experiments 1, 2 and 3 sham operations, in which the adrenals were exposed, were carried out on the control animals. In experiments 4 and 5 the control rats were etherized but not operated.

In experiments 1, 2, 4 and 5 urine collections were commenced without previous fasting. The rats in experiment 3 had been fasted for 1 day. Observations were begun immediately after adrenalectomy except in experiment 5 in which the fasting was commenced 7 days after the opera-

tion. In experiments 1 and 2 each rat received 1 cc. of 10 per cent sodium chloride solution by subcutaneous injection each day. The animals in experiment 3 were given twice daily by stomach tube 3 cc. of a solution containing 0.6 per cent sodium chloride and 0.4 per cent sodium acetate. All rats were allowed distilled water *ad lib* during fasting except those in experiments 4 and 5 which were provided with a 1 per cent sodium chloride solution in its place.

On the days indicated (*) the rats in experiments 1 and 2 received 1 cc. per day and in experiment 3, 2 cc. per day subcutaneously of a commercial preparation (furnished by The Wilson Laboratories under the name of "Phyone") of the anterior pituitary growth hormone extract prepared according to Van Dyke and Wallen-Lawrence (13). The ketogenic extract used in experiments 4 and 5 in the amount of 2 cc. per rat per day was prepared from powdered, acetone dehydrated, anterior pituitary gland in a manner practically identical with that used by Best and Campbell (14).

Discussion. As can be seen from experiments 2 and 1 (all of the adrenalectomized rats in the latter died on the 4th and 5th days) the young rats did not stand the adrenalectomy as well as the older ones which is in line with the usual relation between age and survival (15). Both pituitary extracts were quite toxic in that the survival period of the adrenalectomized rats which received them was usually much shorter than of those which were given only salt solution.

A perusal of the typical experiments in table 1 as well as our other data shows that anterior pituitary extracts which are ketogenic in normal rats fail to produce a ketonuria in rats which have been adrenalectomized. There is an occasional exception to this conclusion such as the rat in the extract-adrenalectomized group of experiment 3 which was responsible for the significant ketonuria recorded in the average result. After the fourth day of fasting these three rats were returned to the stock diet and two died within 4 days. The third, which had the ketonuria following the anterior pituitary extract, survived until killed three weeks later. As in many animals no accessory adrenal cortex tissue was found at autopsy but we believe the ketonuria, like the survival, was due to incomplete removal of cortical tissue. We are assuming here that the cortical tissue is the part of the adrenal the removal of which abolishes the ketogenic response largely because of Evans' results (7). There is other evidence of a more indirect nature in the ketogenic action of adrenal cortex extract (16). In a preliminary report which has appeared since the completion of these experiments Fry (17) reaches very similar conclusions. The question of whether the absence of the adrenal cortex tissue which is stimulated by anterior pituitary extracts to produce an increase in hormone formation or simply the absence of a normal concentration of cortical hormone from the tissues is responsible for the abolition of this ketosis is still uncertain. The

TABLE 1

The influence of adrenalectomy upon the ketogenic activity of anterior pituitary extracts

Ketone body excretion in milligrams of acetone per rat per day

EXPERIMENT NUMBER	NUMBER OF RATS	DAY	CONTROLS				ADRENALECTOMIZED			
			Salt solution		Anterior pituitary extracts		Salt solution		Anterior pituitary extracts	
			Body weight	Acetone	Body weight	Acetone	Body weight	Acetone	Body weight	Acetone
			grams	*mgm.*	*grams*	*mgm.*	*grams*	*mgm.*	*grams*	*mgm.*
1	3	1	114	0.6	113	0.5	115	0.3	117	0.2
		2		1.7		66.7*		1.0		1.8*
		3		18.0		81.2*		6.5		9.2*
2	3	1	94	1.0	95	1.2	95	0.2	95	0.3
		2		0.4		67.0*		0.5		1.0*
		3		0.5		60.0*		1 dead		2 dead
3	3	1	166	14.8	167	43.9*	169	5.5	171	0.7*
		2		29.6		101.2*		37.3		30.1*
		3		35.3		101.9*		6.2		6.3*
		4		39.6		147.5*		4.8		50.2*
4	1(1)	1	250	2.3	210	2.2	228	2.5	222	2.8
		2		2.8		36.4*		1.2		2.2*
		3		1.8		15.1*		1.6		14.7*
		4		1.9		7.8*		1.2		3.6*
	1(2)	1	202	2.3	246	2.2	210	2.5	204	2.8
		2		2.0		100.1*		2.0		5.2*
		3		1.2		264.3*		1.9		17.4*
		4		0.7		14.6*		9.3		0.3*
	1(3)	1	200	2.3	186	2.2	210	2.5	196	2.8
		2		1.7		150.3*		2.5		2.7*
		3		1.9		56.8*		2.5		0.7*
		4		1.5		63.8*		19.6		1.4*
5	1(1)	1					220	2.2	210	1.2
		2						1.7		2.7*
		3								1.3*
		4						1.0		0.4*
	1(2)	1					186	1.7	210	1.9
		2						1.2		0.1*
		3						0.4		1.3*
		4						0.2		1.0*
	1(3)	1					210	2.6	206	1.0
		2						0.7		2.2*
		3						1.0		0.8*
		4						0.5		0.6*

* Days on which anterior pituitary extract was administered.

immediate inactivity of ketogenic anterior pituitary preparations following removal of the adrenals makes the latter possibility most unlikely. The unavailability of a truly satisfactory cortical extract as well as inadequate knowledge of the normal requirement makes it impossible to definitely examine this point at the present time. Most of our experiments were carried out immediately following adrenalectomy both because such animals stand fasting and other procedures better than when they have been maintained on a high salt intake or cortical extract for some time after operation and because only in this way, may one be certain of obtaining chiefly the effects of adrenalectomy *per se* without any of the secondary changes incident to the removal of these glands. However, after a week on a high sodium chloride diet following adrenalectomy we still find that ketogenic anterior pituitary extract produces no ketosis (expt. 5, table 1)

TABLE 2

Ketone body concentration in milligrams of acetone per 100 cc. whole blood in female rats weighing 180 to 210 grams, after fasting 48 hours, and 24 hours after adrenalectomy

Killed 2.5 hours after half the animals were given 3 cc. of anterior pituitary extract.

NUMBER	CONTROLS		ADRENALECTOMIZED	
	Physiological salt solution	Anterior pituitary extract	Physiological salt solution	Anterior pituitary extract
1	2.1	14.2	1.1	4.5
2	1.3	10.6	2.3	4.8
3	1.9	18.2	1.2	14.4
4	4.0	11.5	2.1	4.3
5		20.7		

unless there is evidence of cortical tissue remnants in long survival without therapy.

A possible view would be that in the absence of the adrenal cortex there is an increased destruction of ketone bodies rather than any change in the rate of formation. Direct evidence on this point is now being collected. In table 2 are presented brief data on the ketonemia due to an anterior pituitary extract as influenced by adrenalectomy and although the removal of the glands greatly reduces the rise in the blood ketones and in general confirms the conclusions reached from observations of the ketonuria the extract produced some rise in the adrenalectomized rats. This could be due to increased utilization of ketone bodies which were being produced in quantities higher than usual, a circumstance which might not be at all noticeable in the excretion rate of urine ketones.

Fasting ketosis. We have found (table 1) that removal of the adrenals usually reduces the low ketonuria of fasting rats still lower. An attempt

was made to determine the influence of adrenalectomy on simple fasting ketosis when this is of a higher order of magnitude. Ten adult female rats were fed on a mixture of 50 per cent butter and 50 per cent casein for 3 days and then fasted. It is possible, particularly if the occurrence of ketosis is related to the deposition of fat in the liver (18), that better results would have been obtained with a low protein diet of fat and sugar (19). However a good ketosis was obtained in most of the rats (table 3). Three of the five controls had a very large degree of ketosis. Only one of the adrenalectomized rats had a ketosis within this range and this animal

TABLE 3

The influence of adrenalectomy upon the fasting ketosis of female rats

Ketone body excretion in milligrams of acetone per rat per day

NUMBER	BODY WEIGHT	DAY OF FASTING				
		1	2	3	4	5
Control group						
	grams					
1	160	1.7	49.4	133.0	92.6	6.6
2	165	1.4	17.9	131.0	61.6	133.0
3	138	1.5	3.7	45.2	172.0	128.0
4	152	2.1	1.2	1.8	2.6	14.2
5	170	1.7	1.8	1.7	1.9	2.4
Average..	157	1.7	14.8	62.5	84.1	57.0
Adrenalectomized group						
1	163	1.4	20.0	77.0	46.0	29.3
2	154	0.5	17.0	7.0	4.3	3.8
3	178	1.5	5.2	3.8	1.3	0.5
4	167	1.4	0.8	1.3	0.8	1.2
5	140	1.4	1.8	2.0	0.6	
Average..	160	1.6	8.9	18.2	10.6	8.7

survived the experiment for several weeks without treatment, evidence that there was adrenal tissue not removed at operation. The average figures show a marked diminution in fasting ketosis due to adrenalectomy when this occurs at a high level (table 3).

SUMMARY

Fasting ketosis in the female rat when at a low level following a stock diet or at a high level following fat feeding is reduced by bilateral adrenalectomy.

Ketosis due to ketogenic anterior pituitary extracts as a rule is reduced

or does not occur after the removal of the adrenal glands. When this preparation shows ketogenic properties in adrenalectomized rats they nearly always survive for a long time after operation without therapy, evidence that removal of adrenal cortex tissue was not complete.

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THE REVERSAL BY PROGESTIN OF RESPONSES OF THE NON-PREGNANT UTERUS OF THE CAT

J. H. KENNARD

From the Department of Physiology in the Harvard Medical School

Received for publication September 12, 1936

The responses of the cat's uterus to stimulation of the hypogastric nerves, or to injections of adrenine, change from relaxation in the non-pregnant animal to contraction during pregnancy. A similar reversal may be induced by injections of progestin (Van Dyke and Gustavson, 1929). It was thought that a study of the temporal course of the reversal of the responses to these two stimuli might yield information with respect to the adequacy of current theories on the chemical transmission of sympathetic nerve impulses. The present paper deals with such a study.

METHOD. Mature, non-pregnant female cats, spayed 3 to 10 days beforehand, were injected intraperitoneally with various amounts of progestin.[1] Three to twenty-four hours later the animals were anesthetized (dial, Ciba, 0.7 cc. per kgm. intraperitoneally). A median lower abdominal incision was then made, and electrodes placed on the hypogastric nerves. One horn of the uterus was dissected free at its tubal end, and attached by a thread to a recording lever. For nerve stimulation a Harvard inductorium was used, with 10 volts in the primary circuit. Tetanizing stimuli were applied for 10 seconds, unless otherwise stated. For stimulation by adrenine, a solution of adrenalin chloride, 1/100,000, was injected into the femoral vein in amounts of from 0.1 to 0.5 cc. (1 to 5γ). The responses were arbitrarily divided into four categories, defined as follows:

(−) Minus: a marked relaxation (figs. 1B and 2B).

(+) One plus: a diphasic reaction consisting of a contraction followed by a more marked relaxation (fig. 1A). This reaction is predominantly a relaxation, i.e., the relaxation is quantitatively greater than the contraction.

(++) Two plus: a similar diphasic reaction in which the contraction is more marked than the relaxation.

(+++) Three plus: a marked contraction (fig. 2A and C). This type of reaction may be followed by a brief period of inhibition of spontaneous

[1] The progestin used was of two types, one a natural product (Progestin 6991-2-B, generously furnished by the Upjohn Co.), the other synthetic (Proluton, Schering). Both gave similar results.

contractions, but there is no relaxation below the base line of normal contractions.

RESULTS. A total of 21 cats, including controls, were used in these experiments; 17 received progestin, and of these, a reversal of the response to nerve stimulation took place in 14. This reversal invariably preceded any change in the responses to adrenine by several hours (fig. 1). By injecting the minimal amount of progestin effective to reverse the nerve response, it was found possible to do so without altering the adrenine response.

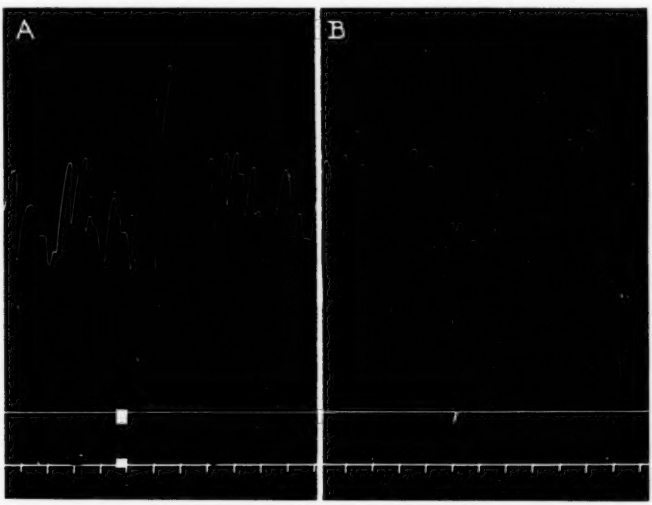

Fig. 1. Non-pregnant cat (1.7 kgm.), 4 days after spaying. "Proluton" (1 unit) injected 9 hours previously. Responses of the uterus: A, to 10-second stimulation of the hypogastric nerves (coil distance 8 cm.); B, to adrenalin (5γ).

In a single animal, after the injection of progestin, a constant record was not taken from before the beginning of the reversal until the reactions once more returned to normal. The various stages have been followed, however, in different animals. By piecing together selected cases from the group, it is possible to reconstruct what probably happens in a single animal.

Following the injection of a suitable dose of progestin, for example, 0.4 to 1.0 mgm. progesterone per kilogram, there is a latent period of 3 to 6 hours before demonstrable change takes place in the uterus. At the end of this latent period the response to hypogastric nerve stimulation slowly changes from − to +. Within 6 to 12 hours, i.e., 9 to 18 hours after in-

jection, the response to nerve stimulation will have become a full +++. Up to this time there will have been no change in the response to adrenine, which will still be − in spite of the + reactions to nerve stimulation (fig. 2). Shortly thereafter the response to adrenine will become diphasic (first +, then ++), provided that sufficient progestin has been administered; during the next 4 to 10 hours the adrenine response will also become +++. The reversal of the response to adrenine takes place much more slowly, and it may be more than 24 hours before the maximum is reached.

After a period of time the effect of the progestin wears off, and the responses to both adrenine and nerve stimulation again become −. The time relations of this phase are varied, depending apparently not only on the dosage of progestin, but also on the condition of the animal. In some cases the response to nerve stimulation parallels the adrenine response, as both return through ++ and + to − together; in other cases, particularly when only just sufficient progestin has been administered to reverse the adrenine response, the adrenine response will return to − considerably before the nerve response.

As stated before, if insufficient progestin has been administered to reverse the adrenine response, one may reverse the nerve response alone. Provided that the dosage is exactly right, one may produce a full +++ response to nerve stimulation, accompanied by a − response to adrenine (fig. 2). If a slightly greater amount of progestin is given, the adrenine response will become diphasic, but only after the nerve response has begun to diminish. The adrenine response may change from + to ++ as the nerve response changes from +++ to ++, both returning through + to − simultaneously.

Summarizing, the reversal takes place first in the nerve response, and only considerably later, if at all, in the adrenine response. In some cases the return to normal is simultaneous, while in others the adrenine response returns to normal considerably ahead of the response to nerve stimulation.

When the responses to adrenine were −, and the responses to tetanizing stimulation for 10 seconds of the hypogastric nerves were +++, it was found possible to demonstrate some degree of relaxation and an inhibition of spontaneous contractions if the stimulus was continued over a more extended period of time (30 seconds to 2 minutes). In other words, as long as the adrenine response remained −, a slight − element could be demonstrated in the nerve response by a longer period of nerve stimulation.

Discussion. The data (figs. 1 and 2) show that in the cat, under the prescribed conditions, the uterus will contract in response to stimulation of its sympathetic nerves, and yet relax in response to stimulation by adrenine. This fact must be considered in the formulation of theories concerning the chemical mediation of sympathetic nerve impulses.

The possibility suggested itself that the discrepancies between the

responses to the nerve impulses and the responses to adrenine might be accounted for by cholinergic fibers in the hypogastric nerves of the cat. That this factor was not of any considerable importance was demonstrated by paralyzing the cholinergic fibers by a suitable dose of atropine. This treatment was found not to have any material effect upon the configuration of the response to nerve stimulation. It was therefore concluded that the response to nerve stimulation was brought about by impulses from true adrenergic nerves.

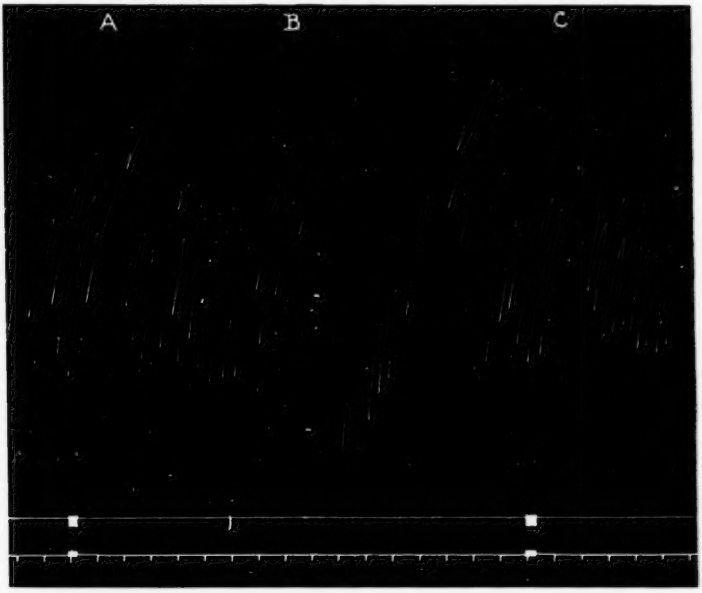

Fig. 2. Non-pregnant cat (2 kgm.), 4 days after spaying. "Prolaton" (1 unit) injected 13 hours previously. Responses of the uterus: A and C, to 10-second stimulation of the hypogastric nerves (coil distance 6 cm.); B, to adrenalin (5γ).

It is generally considered as an established fact that impulses from sympathetic adrenergic nerves are mediated by the liberation of an adrenine-like substance (Cannon, 1934; Bacq, 1935). According to this theory stimulation of the hypogastric nerves in the cat would cause the liberation of adrenine within the myometrium. If this is true, how is it possible for such stimulation to bring about a response differing qualitatively from the response to adrenine? Given that this difference may exist, one is forced to consider the following alternatives.

First, the substance liberated by the nerve impulses may not be adrenine.

This hypothesis is contrary to the evidence of most of the work in this field, and has few, if any, observations to support it. However, if the mediator differs slightly from adrenine, it might have a different effect on an organ in a state of transition, and this would adequately explain the phenomenon described.

Another alternative is that the substance liberated at the nerve endings is adrenine, but that it becomes modified before acting on the smooth muscle cells (Cannon and Rosenblueth, 1933). Probably only a small per cent of these smooth-muscle cells are innervated. It is thought that stimulation of the nerves causes liberation of adrenine within the innervated cells, and this mediator diffuses from these, stimulating the adjacent non-innervated elements.

It is the contention of Cannon and Rosenblueth (1933) that the liberated adrenine (M) combines with a receptive substance (H) present in the cell to form a compound (MH). This hypothetical H substance may be either excitatory (E) or inhibitory (I). If the cell contains E, the compound will be sympathin E (ME) and the cell will contract in response to nerve stimulation; if the cell contains I, the compound will be sympathin I (MI) and the cell will relax. Theoretically, in the normal non-pregnant cat, the uterine muscle cells contain I and therefore relax. When pregnancy takes place, the progestin in the blood stream in some way causes the I to be converted into E. During this conversion there will be present both I and E, and nerve stimulation will therefore give a diphasic reaction. If each cell contained a similar proportion of I to E, then presumably nerve stimulation and stimulation by adrenine would cause similar responses.

Among the slight physiological differences between innervated and non-innervated cells, there may be some difference which affects the reaction of that cell to progestin. If this were true, then the reaction $I \rightarrow E$ might take place earlier or more rapidly in the innervated cells. Let us suppose this to be the case, and that by giving the proper amount of progestin we have brought about a state in which all cells contain both E and I; the distribution would not be uniform, for cells receiving nerve endings would contain predominantly E, non-innervated cells predominantly I. The non-innervated cells outnumber the innervated cells. Now let us suppose that we administer adrenine, via the blood stream. This adrenine affects all cells simultaneously. The innervated cells contract, while the non-innervated cells relax. Because of numerical proportions the sum of these two reactions would be a relaxation as recorded on the kymograph. Again, let us suppose that we stimulate the nerves. In this case the nerve endings liberate the adrenine within the innervated cells, which contain predominantly E. The liberated adrenine combines immediately with the E to form the compound sympathin E. The innervated cells contract. The diffusing substance in this case is not adrenine but sympathin E, which

has been shown by Cannon and Rosenblueth (1933) not to be capable of causing relaxation of the cat's uterus. Therefore, if any reaction to the diffusing sympathin E takes place, it must also be in the nature of a contraction, and the sum of the reactions, as recorded on the kymograph, would be a contraction.

The observation, that when the two responses are opposite an element of relaxation can be demonstrated in the responses to the nerves after prolonged stimulation (p. 192), can be easily explained in accordance with this theory: a long-continued stimulation causes the liberation of an excess of the mediator M, all of which does not combine with the H substance in the innervated cells. Therefore, the excess M diffuses outward along with the sympathin E and is free to act on the other cells, which contain I. It produces, therefore, the − element in the reaction.

Let us now consider the same phenomena from the point of view of those who do not subscribe to the theory of Cannon and Rosenblueth concerning the existence of sympathin E and sympathin I. According to Bacq (1935) the substance described by Cannon and Rosenblueth as sympathin E may merely be a modified adrenine, probably an intermediate metabolite such as an oxidation product, which retains only some of the physiological properties of adrenine. If one subscribes to this theory then obviously the metabolism of the liberated adrenine must take place within the innervated cells, and the modified products then diffuse to the other cells. It is difficult, however, to explain why these cells modify the liberated adrenine without exerting a similar effect upon adrenine brought to them in the blood stream. Diffusion of the excess adrenine following long-continued stimulation is equally acceptable according to either theory.

SUMMARY

Injections of progestin reverse from relaxation to contraction the responses of the uterus of the non-pregnant cat to stimulation of the hypogastric nerves and to injections of adrenine. The reversal of the responses to nerve stimulation precedes that of the responses to adrenine. For a period of time the uterus responds to nerve stimulation by contraction and to adrenine by relaxation (figs. 1 and 2). The significance of these observations in relation to current theories on the chemical transmission of sympathetic nerve impulses is discussed.

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CARBOHYDRATE STORAGE AND MAINTENANCE IN THE HYPOPHYSECTOMIZED RAT[1]

JANE A. RUSSELL AND LESLIE L. BENNETT

From the Institute of Experimental Biology, University of California, Berkeley

Received for publication September 14, 1936

In spite of the relationship now known to exist between the pituitary and certain aspects of carbohydrate metabolism, almost nothing has been known until recent months of the effects of hypophysectomy alone on carbohydrate metabolism per se. As the rat has been the subject for extensive studies of other functions of the hypophysis, and as its normal carbohydrate metabolism is also well known, we have chosen it as a suitable species for this study.

It was reported by Phillips and Robb (31) and subsequently confirmed by us (33) that fasted hypophysectomized rats have low levels of blood glucose and of muscle and liver glycogen. As preliminary findings indicated that the hypoglycemia and low glycogen levels might be caused by an abnormal rate of depletion of carbohydrate reserves during fasting, the following experiments were undertaken:

1. A comparison under standardized conditions of the carbohydrate levels of normal rats, of partially hypophysectomized rats, and of rats completely hypophysectomized for various lengths of time.

2. A study of the carbohydrate levels of these animals as a progressive effect of fasting as contrasted with non-fasted controls.

3. The preparation of curves showing the changes in carbohydrate levels in these animals following the feeding of known amounts of carbohydrate.

If an abnormal rate of depletion of carbohydrate reserves during fasting were to be attributed definitely to loss of the anterior pituitary in these animals, then besides affording a possible explanation of the spontaneous hypoglycemias known to occur in other species of hypophysectomized animals, this finding would also establish a definite point of attack upon the main problem of the rôle of the pituitary in carbohydrate metabolism.

[1] Aided by the Presnell Fund for the study of the metabolic relations of the anterior hypophysis, contributed by Robert R. Presnell, Frank Tuttle, Arthur Stebbins and Mrs. Gordon Kahn, of Los Angeles.

METHODS. All rats were young males in good condition, and, unless otherwise specified, in uniform weight and age groups as follows:

	AGE	BODY WEIGHT (FASTED)	TIME POST-OPERATIVE
	days	*grams*	*days*
Hypophysectomized	65–80	130–160	20–30
Operated controls	60–70	200–230	16–20
Normal controls (1)	47–50	140–160	
Normal controls (2)	65–70	200–230	

No differences were found between sub-groups (1) and (2) of the normal controls, so that all figures are included in one series of averages.

The operation was performed by the parapharyngeal route, and the results checked at autopsy in all cases. Animals included in the group of operated controls were partially hypophysectomized, all of the posterior lobe being removed, but one-third or more of the anterior lobe remaining in situ.

Samples of muscle, blood and liver were taken under amytal anesthesia with the usual precautions. The whole livers were removed in all cases, so that liver glycogen values expressed as milligram per 100 grams of rat (in tables 2 and 6) were calculated directly for each determination.

Analyses of glycogen were made by the method of Good, Kramer and Somogyi (14) as modified by Cori and Cori (10). The final sugar determinations were made with the Shaffer-Somogyi reagent (35). Liver glycogen figures include only fermentable reducing substances (after two precipitations). Muscle glycogen values for each animal were averages of two separate determinations of total reducing substances in the two gastrocnemii.

The carbohydrate meals were given in the form of cornstarch, in 50 per cent suspension in water, fed by stomach tube without anesthesia. When a metal collar was used on the syringe, deliveries of hydrolyzable carbohydrate were checked within 1 per cent. Starch was fed rather than glucose because the large quantities of carbohydrate desired in these experiments when given in solution as glucose, caused diarrhea and irregularities in absorption in the hypophysectomized rats. When the rate of utilization of the starch was determined, the whole gut of each animal was washed well in warm water, the contents digested in hot 1 N HCl for three hours, and the fermentable carbohydrate in the hydrolysate determined by the usual methods. In check experiments, the recovery of carbohydrate from the gut immediately after feeding was practically complete.

The diet of the rats previous to the experiments was a uniform mixture containing 67 per cent wheat.[2] It maintained the hypophysectomized

[2] The diet consisted of 67 per cent ground whole wheat, 5 per cent fish oil, 10 per cent fish meal, 5 per cent casein and 10 per cent alfalfa.

rats in good condition without additional supplement of carbohydrate. In certain experiments the amount of food allowed normal rats was restricted to that just sufficient to maintain the body weight during the

TABLE 1

Carbohydrate levels of hypophysectomized and control rats

	NUMBER OF ANIMALS	BLOOD GLUCOSE	LIVER GLYCOGEN	MUSCLE GLYCOGEN
		mgm. per cent	*mgm. per cent*	*mgm. per cent*
A. Not fasted:				
1. Hypophysectomized	9	124 ± 3	1780 ± 370	590 ± 25
2. Unoperated	12	125 ± 1	1650 ± 100	530 ± 9
3. Partially hypophysectomized	6	123 ± 8	2070 ± 160	530 ± 33
B. Fasted 8 hours:				
1. Hypophysectomized	7	60 ± 2	17 ± 1	445 ± 15
Hypophysectomized	11	82 ± 3	109 ± 23	468 ± 10
2. Unoperated	6	100 ± 3	1210 ± 210	490 ± 16
Unoperated	12	131 ± 2	1660 ± 140	514 ± 8
3. Partially hypophysectomized	7	109 ± 3	1860 ± 190	515 ± 12
C. Fasted 16–18 hours:				
1. Hypophysectomized	10	57 ± 3	18 ± 1	350 ± 14
2. Unoperated	12	85 ± 3	38 ± 4	500 ± 8
3. Partially hypophysectomized	10	103 ± 3	71 ± 53	480 ± 12
D. Fasted 24 hours:				
1. Hypophysectomized	7	42 ± 2	13 ± 1	354 ± 10
Hypophysectomized	10	50 ± 1	15 ± 1	322 ± 8
2. Unoperated	6	71 ± 2	26 ± 3	529 ± 4
Unoperated	11	80 ± 2	23 ± 1	502 ± 5
3. Partially hypophysectomized	9	85 ± 2	27 ± 4	429 ± 7

TABLE 2

*Approximate loss of carbohydrate during 24 hours of fasting in milligrams per 100 grams body weight**

	AS BLOOD GLYCOGEN	AS LIVER GLYCOGEN	AS MUSCLE GLYCOGEN	TOTAL
Normal	25	73	9	107
Hypophysectomized	38	62	120	225
Control-operated	18	74	50	142

* Calculated from figures in table 1, assuming blood glucose distributed in and muscle content equal to 50 per cent of the body weight.

experimental periods. This was 7 to 9 grams of the diet, only slightly more than voluntarily consumed by hypophysectomized rats.

During all fasting periods the rats were kept in small cages with wire screen bottoms at an approximately uniform temperature (75°F.).

RESULTS. The values obtained for the carbohydrate levels of standard

series of normal and hypophysectomized rats are summarized in table 1. As can be seen, the levels of the body carbohydrates determined in the hypophysectomized animals when they were in the usual fed state were entirely normal. In the fasted rats, on the other hand, an abnormal decrease was apparent in blood glucose and liver glycogen in as short a time as eight hours of fasting, and by sixteen hours the loss of body carbohydrate in the completely hypophysectomized animals was striking. Although the actual percentage changes in muscle glycogen were not large, the loss of total body glycogen was considerable. This fact is brought out in table 2.

Although these figures can be only roughly approximate, they show that, in spite of the lower metabolic rate of the hypophysectomized rats, the total disappearance of carbohydrate in these animals during fasting was markedly greater than in the normal rats.

That this effect of hypophysectomy was not due to removal of the posterior pituitary was shown by the fact that the carbohydrate levels

TABLE 3

Carbohydrate levels of normal fasted rats kept on restricted food intake for 10 to 20 days

	NUMBER OF RATS	BLOOD GLUCOSE	LIVER GLYCOGEN	MUSCLE GLYCOGEN	
		mgm. per cent	*per cent*	*per cent*	
18 hours after last meal.....	4	131	4.23	0.62	Gut still contained food
42 hours after last meal.....	4	114	0.21	0.48	Gut empty

determined in the partially hypophysectomized series, with one-third or more of the anterior lobe present, were practically normal. Operative injury to the hypothalamus was not a factor, since in several of the control-operated rats, puncture of the diaphragma sella and injury to the subjacent brain were also made without affecting the results.

Since completely hypophysectomized rats usually eat much less than nomal rats on the same diet (about half, in these experiments), the effects of chronic inanition might have been a factor in the results. However, when normal rats were kept on a restricted diet for a period of ten to twenty days, they suffered no impairment in their ability to maintain their carbohydrate stores during subsequent fasting. The results of some of these experiments are presented in table 3.

In spite of the consistent finding of glycogen reserves within normal limits in unfasted hypophysectomized rats, and marked depletion of these stores after short fasts, objection may be made to the use of rats simply taken from their cages for determinations of the fed levels and to start the fasting periods. Therefore, an attempt was made to obtain uniform

high carbohydrate stores by feeding known amounts of starch and to follow the course of the decreases during the subsequent fasting period. The average figures obtained are presented in table 4.

For reasons which will be discussed later, the attempt to obtain high uniform carbohydrate levels was not entirely successful, the hypophysectomized rats failing markedly to deposit as much liver glycogen as did

TABLE 4

Carbohydrate levels of normal and hypophysectomized rats during fasting following uniform feeding

One gram starch fed to male rats weighing 140–160 grams after 16–18 hours fast

	NUMBER OF RATS	BLOOD GLUCOSE	NUMBER OF RATS	LIVER GLYCOGEN	NUMBER OF RATS	MUSCLE GLYCOGEN
		mgm. per cent		*per cent*		*per cent*
A. Normal control rats						
Before feeding	13	85 ± 3	10	0.04 ± 0.004	12	0.50 ± 0.01
After feeding:						
4 hrs.	8	138 ± 4	8	2.56 ± 0.06	8	0.69 ± 0.02
8 hrs.	6	128 ± 2	9	1.74 ± 0.11	5	0.59 ± 0.01
12 hrs.	6	133 ± 2	6	1.14 ± 0.11	4	0.61 ± 0.02
24 hrs.	6	119 ± 3	6	0.45 ± 0.09	5	0.55 ± 0.01
36 hrs.	5	87 ± 7	6	0.05 ± 0.01	5	0.57 ± 0.02
B. Hypophysectomized rats						
Before feeding	10	57 ± 3	8	0.018 ± 0.001	10	0.35 ± 0.01
After feeding:						
4 hrs.	9	111 ± 5	9	0.49 ± 0.08	7	0.55 ± 0.03
8 hrs.	10	82 ± 4	10	0.21 ± 0.05	5	0.46 ± 0.02
12 hrs.	7	46 ± 3	7	0.022 ± 0.003	5	0.48 ± 0.01
24 hrs.	5	54 ± 2	5	0.014 ± 0.001	5	0.41 ± 0.02
C. Hypophysectomized rats fed 2 grams starch (1 gm. 4 hrs. following first)						
After feeding first time:						
8 hrs.	9	125 ± 4	10	1.24 ± 0.08	8	0.57 ± 0.03
12 hrs.	6	87 ± 6	6	1.22 ± 0.30	5	0.52 ± 0.01
25 hrs.	6	67 ± 6	6	0.03 ± 0.01	6	0.44 ± 0.01
36 hrs.	5	40 ± 3	5	0.010 ± 0.001	5	0.38 ± 0.01

the normal animals when fed the same amount. However, when an additional amount of starch was later fed to the operated rats, in eight hours after the first feeding (four hours after the second), all carbohydrate levels approached those found in normal rats eight hours following their first feeding.

Yet, in spite of the fact that absorption must still have continued in

the operated rats for some time past the eight hour period (when the carbohydrate levels were approximately equal to the normal), and that during the whole experimental period the hypophysectomized rats must have disposed of twice as much carbohydrate, subsequently all the operated animals were found to have carbohydrate levels much below those of the normals. Therefore, although these data cannot be given a strictly quantitative significance, they do present graphically the inability of the hypophysectomized rat to maintain its carbohydrate stores during fasting.

The apparent failure of the hypophysectomized rats to form glycogen from fed carbohydrate, while at first sight presenting another abnormality of carbohydrate metabolism in these animals, appears on closer analysis not so significant. In the first place, as shown by the effects of double feeding and by the findings in unfasted animals, the hypophysectomized rats, if given sufficient time and raw material, are able to form normal glycogen reserves. On this evidence, the ability per se to deposit glycogen would not seem to be more than somewhat decreased in rate by general tissue inactivity.

Secondly, the marked defect in glycogen deposition immediately after feeding may be largely explained by known factors. The rate of intestinal absorption of glucose is known to be low in hypophysectomized rats (Phillips and Robb, 31; Bennett, 3) and the rate of digestion and absorption of starch is also decreased by about one-third:

TABLE 5

Carbohydrate absorbed during 4 hours after starch feeding

	NUMBER OF RATS	RANGE	AVERAGE
		mgm./100g/hr.	*mgm./100g/hr.*
Normal	4	111–137	127
Hypophysectomized	4	85–108	92

Now, as is shown in table 6, the average total amount of carbohydrate accounted for after feeding is also much decreased, but as this decrease is roughly of the same proportion as that of the absorption rate, no abnormal disappearance of absorbed carbohydrate is indicated by these figures.

The deposition of liver glycogen after feeding of starch (or glucose) to the hypophysectomized rat still appears markedly abnormal, even when account is taken of the decrease in absorption rate. However, it must be borne in mind that the very low initial levels of tissue sugar and especially of muscle glycogen would, during the early period after feeding, cause the uptake of a large part of the absorbed glucose in these tissues at the expense of liver glycogen. These facts are brought out in table 6 in which it is seen that during the first four hour period following feeding in the hypo-

physectomized rats, 90 per cent of the body carbohydrate accounted for was found in muscle and tissue fluid as compared to 50 per cent of the normal, although the carbohydrate levels in these tissues never equaled those in the normal under the same conditions. This factor, that of low initial fasting levels, might alone cause a marked depression of liver glycogen deposition, and together with the low absorption rate, appears to account for most of the abnormal disposition of fed carbohydrate in hypophysectomized rats. But the operation of other possible factors is not ruled out. If, as found by Fisher and Pencharz, a larger proportion of carbohydrate were oxidized by hypophysectomized rats following glucose feeding, this process also would be expected to decrease the amounts of glycogen stored in the liver under these conditions.

In all of the experiments so far presented, the hypophysectomized rats were not used until from three to four weeks after the operation in order to rule out any immediate post-operative effects. A series of determinations

TABLE 6

Approximate increases in body carbohydrate of rats four hours following starch feeding, calculated from figures in table 4 (on same basis as in table 2)

	AS BLOOD GLUCOSE	AS LIVER GLYCOGEN	AS MUSCLE GLYCOGEN	TOTAL
Normal:				
Mgm./100 grams body weight	29	109	85	223
Per cent of total	13	49	38	
Hypophysectomized:				
Mgm./100 grams body weight	27	16	100	143
Per cent of total	19	11	70	

of fasted carbohydrate levels was also made on hypophysectomized animals at various intervals after the operation in order to place the time at which the maximum effects became evident. The results of these series, controlled by sham operations entirely like the real ones except that the glands were undisturbed, are presented in table 7. Surprisingly, the inability to maintain carbohydrate stores during fasting appears practically fully developed within 24 hours following the removal of the pituitary, in contrast to the considerable length of time usually necessary for the full development of metabolic effects following thyroidectomy or adrenalectomy in these animals.

Discussion. Although the blood sugar levels in hypophysectomized animals under ordinary conditions are usually found to be normal or only slightly low ((5, 7, 16, 20, 21, 23, 32, 34) in dogs, (27) in cats, (37) in toads, and (29) in dogfish). It is also a well established observation that these animals have a definite tendency toward hypoglycemia. This effect is sometimes reported as chronic ((2, 6, 11, 22, 25) in dogs, (13) in rabbits,

(15) in birds, (17) in toads), but more often it appears in individual attacks, commonly as a result of fasting, ((4, 7, 21, 25, 26, 36) in dogs, (7, 26) in monkeys, (9) in rabbits, (30) in guinea pigs, (31) in rats). It is now recognized that fasting is an essential factor in the production of hypoglycemia in hypophysectomized animals (7, 19).

Liver and muscle glycogen levels, determined in several species of hypophysectomized animals have also generally been found to be normal when these animals were otherwise normal ((1, 5, 16) in dogs, (8, 9, 28) in rabbits, (18) in toads). This fact, however, is not necessarily inconsistent with

TABLE 7

Carbohydrate levels in fasted hypophysectomized rats at various intervals after operation

Male rats 46–54 days of age, weight 130–180 grams fasted

	NUMBER OF ANIMALS	BLOOD GLUCOSE	LIVER GLYCOGEN	MUSCLE GLYCOGEN
		mgm. per cent	*mgm. per cent*	*mgm per cent*
Fasted 24 hours:				
A. Unoperated controls	11	80 ± 2	23 ± 1	502 ± 5
B. Sham-operated controls 24 hours post-operative	2	78	20	493
C. Hypophysectomized:				
24 hours post-operative	6	65 ± 2	18 ± 3	363 ± 12
2 days post-operative	4	58	22	389
3 days post-operative	5	66	19	310
5 days post-operative	3	53	22	293
11 days post-operative	5	51	23	302
21 days post-operative	10	50 ± 1	15 ± 1	322 ± 8
Fasted 8 hours:				
A. Unoperated controls	12	131 ± 2	1660 ± 140	514 ± 8
B. Sham operated and restricted food intake. 24 hours post-operative	4	115	154	604
C. Hypophysectomized:				
24 hours post-operative	5	82	14	340
21 days post-operative	11	82 ± 3	109 ± 23	468 ± 10

the findings presented here, for it must be remembered that larger animals fasted for 24 hours are not necessarily comparable in respect to their metabolism to such small animals as the rat, also fasted 24 hours and that the time relations of many metabolic effects vary widely from species to species. Therefore, it is noteworthy that on the few occasions when liver glycogen values have been determined in conditions of marked hypoglycemia, they also have been very low ((19) in dogs, (9) in rabbits, (17) in toads, (31) in rats), as were also the levels of muscle glycogen determined in two of these cases (17, 30).

The phenomenon here described in rats—the rapid loss during fasting of liver glycogen, followed by hypoglycemia and then by loss of muscle glycogen—is not then opposed to available evidence. Indeed, it seems probable on the basis of the facts mentioned above that if carbohydrate levels in other species of hypophysectomized animals were studied with regard to nutritional states, some such relation as described here in rats would be found to exist in them also.

The possible mechanisms involved in the phenomenon described here are discussed elsewhere, but it may be mentioned that the significance of these observations lies not so much in themselves, but in their relation to the actual rôle of the hypophysis in carbohydrate metabolism; for the other disturbances in carbohydrate metabolism now known to be connected with the pituitary are undoubtedly related in a basic manner to the apparent necessity of the hypophysis in the mechanism by which body glycogen is conserved and blood glucose maintained during fasting.

CONCLUSIONS

1. In the absence of the pituitary, the rat maintains normal levels of liver and muscle glycogen, and of blood sugar when it is fully fed, but when it is fasted, even for very short periods, its carbohydrate levels all are decreased much more than in the normal.

2. The abnormal depletion of carbohydrate stores during fasting is not due to absence of the posterior pituitary, to the effects of brain injury which may be secured in the type of operation used, or to chronic inanition.

3. The feeding of single carbohydrate meals to fasted hypophysectomized rats does not result in body carbohydrate levels comparable to the normal so fed; but this result can be explained in large part, although not necessarily entirely, by the low initial fasting levels and by the low absorption rates existing in these animals.

4. The effect of hypophysectomy on the maintenance of carbohydrate levels during fasting becomes evident within 24 hours after removal of the gland.

The authors wish to thank Dr. Herbert M. Evans for his assistance and counsel in this work.

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